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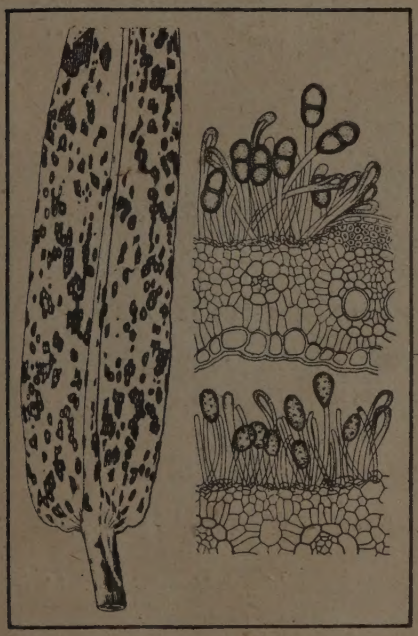

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STUDIES IN LINSEED RUST, MELAMPSORA LINI (PERS.) LÉV. IN INDIA

By RAGHUBIR PRASADA

(Accepted for publication September 20, 1947)

THE largest linseed producing country in the world, after Argentina, is India with five million acres under this crop. A limiting factor in its production is, however, a rust caused by *Melampsora lini* which is codistributed with its host. As the disease does much damage to the crop causing a reduction in yield, breaking off of the stems at the point of attack and even the death of the plants, the losses it annually causes are enormous. The rust has been extensively investigated in several countries and is briefly described by Butler (1918) and mentioned by Howard (1921) but no investigation commensurate with its importance appears to have been undertaken in India, until these studies were started by the writer in 1939.

Linseed (*Linum usitatissimum* L.) is sown in October-November and harvested in March-April in India. The rust first appears, as a rule, in February though Butler (1918) mentions of its having been observed as early as the end of November in parts of Central Provinces. Within a few days of its appearance, most of the linseed fields get rapidly affected. Towards harvest the telia appear and the plants get the characteristic "fired" appearance. The rust is autoecious and is therefore without an alternate host. As there is, so far as can be ascertained, no collateral host on which it can survive during the period April-November and even later, the question of oversummering of the rust has remained a mystery. The mode of its survival from one season to the next is, therefore, obscure.

These investigations were undertaken to throw light on the life history of the rust in India and the problem of its oversummering. The study includes an investigation on the influence of environmental factors on the germination of the uredio- and telio- spores, development of the rust on its host under different environmental conditions and determination of the physiologic races existing within the country.

The Uredial Stage

All the green parts of the plant are attacked. The uredia are minute, scattered, roundish or oblong, sub-epidermal, orange yellow and paraphysate. The urediospores are more or less globose and measure 19—23 x 17—22μ.

Fresh urediospores when placed on a thin film of water start germinating in one hour and twenty minutes and germination progressively improves and the maximum is reached after 12 hours. Since inoculated plants, kept in the moist chamber, get infected in three hours, it is apparent that in nature germination and infection in the presence of a film of water takes place in a relatively short period of time. Germination has been observed to take place at temperatures between 3° and 30° C with optimum at 15—16° C.

Influence of temperature on the viability of urediospores

Waterhouse and Watson (1944) found that urediospores of some physiologic races tolerate much higher temperatures than those of others.

During the course of these studies, infected plants were exposed to different temperatures between 30° and 50° C for 6-24 hours and the urediospores from such plants were then tested for germination at 16—18°C. The results are given in Table I and show that 24 hours' exposure at 38—43°C and 9 hours' exposure at 43–50° C killed all the spores.

TABLE I

Influence of high temperatures (30–50° C) *on the viability of urediospores.* (*The material showed* 90—95 *per cent germination before exposure*)

Temperature	Per cent germination after exposure for			
	6 hours	9 hours	12 hours	24 hours
35-38°C	25	25	15	10
38-43°C	10	8	T	0
43-50°C	T	0	0	0

T=trace, *i.e.*, less than one per cent

Cultures of rust in the uredial stage in the hills and the plains

(i) *Culture in the hills :* A culture of this rust was successfully maintained in the uredial stage in a greenhouse as well as under natural conditions in a miniature plot at Simla (altitude 7,200 ft.) for six years. Plants were also inoculated and kept in the open and in the greenhouse, simultaneously, at different times of the year to determine the influence of weather on the rust and the effect of temperature on the incubation period. The following observations were made.

1. The cultures flourished throughout the year in the greenhouse as well as in the open.

2. The minimum incubation period in the greenhouse as well as in the open was 7 days at 13—21°C.

3. The longest incubation period was 18 days in the open during winter when the temperature range was 2—10°C. Plants inoculated at the same time and kept in the greenhouse at 12—17° C developed rust pustules on the ninth day.

The cultures flourished throughout the year under natural conditions and the continuity of the cultures was never broken. Viability of urediospores collected from the miniature plots was tested every fortnight. The results show that weather in the hills at places like Simla is never unfavourable for the propagation of this rust in the uredial stage and the urediospores are not killed by the heat of summer or the cold of winter.

(ii) *Culture in the plains :* Separate cultures of this rust in the uredial stage were started at Agra, in the plains, in March, 1939, with fresh material received from the crop at Cawnpore, Allahabad and Pusa. The urediospores were transferred to seedlings every three weeks in order to maintain these cultures which flourished fairly well in the greenhouse till the first week of April and under shade in the open till the end of that month. After that, no infection was produced on seedlings that were inoculated on May 4, although, in some cases, ice was put under the moist chamber for 48 hours after inoculation.

From May to October several inoculations were made at Agra with viable urediospores secured from cultures maintained at Simla, but without success. In June even the plants could not bear the heat and were killed. Inoculations were again made on November 29 and they gave positive results. The cultures were then maintained at Agra under natural conditions throughout the winter of 1939-40 and until the end of April. However, with the approach of May, the seedlings failed to get infected. In 1941 also the cultures could not be continued after April 21st.

A collection made from the crop in the plains in April and sent to Simla in June did not show any viable urediospores and no cultures could be established from that sample.

These observations indicate that the urediospores of this rust cannot remain viable and produce infection under conditions prevailing in the plains during May to October.

Results of inoculation experiments carried out at Agra at different times of the year when considered along with the observations made at Simla where the cultures flourished well, even at 30—32° C, indicate that temperature up to 32° C is fairly suitable for infection and rust development.

Longevity of urediospores under storage

A number of factors, but chiefly temperature and moisture, considerably influence the longevity of spores under storage. Peltier (1923) found that urediospores of black rust of wheat retained their viability best at a moderate humidity and low temperature. Humidities of 20 to 40 per cent were found by Bailey (1923) to be optimum for the urediospores of *Puccinia helianthi*, and at temperatures up to 23°C they retained their viability for atleast 185 days. According to Barclay (Arthur 1929, p. 223), urediospores of *Melampsora lini* could remain viable for 75 days. Hart (1926) maintained the viability of urediospores of this rust for seven weeks after storage at 7°C and 60 per cent. relative humidity. Waterhouse and Watson (1944) found that urediospores of certain physiologic races of *M. lini* survived longer periods of artificial storage at low temperatures than those of others.

During these studies spores were kept in sealed glass tubes at 0°, 5—7° and 10—15°C and germination was tested every week. The material gave nearly 100 per cent germination to begin with but there was a gradual decline in viability of the spores at all the temperatures as the period of storage advanced. Detailed results are given in Table II and show that urediospores retain their viability for 18—20 weeks at 5—7°C.

TABLE II

Longevity of urediospores under storage at different temperatures

Weeks of storage	Per cent germination of spores stored at		
	0°C	5-7°C	10-15°C
1	100	90–100	90
2	80	90–100	80–90
3	70	80– 90	60–80
5	30	75	40–60
7	33	50– 60	25–30
11	20	33– 40	5–15
14	T	20– 25	0– 5
16	0	20	0– T
18	0	10	0
20	0	0– T	0
22	0	0	0

T—trace, *i.e.* less than one per cent

THE TELIAL STAGE

The uredial stage is followed by a blackening of the host tissues due to the formation of telia. The teliospores which are massed together and encrusted in the tissues of the host are unicellular and somewhat cylindrical.

Melampsora lini is an autoecious rust and all the stages are found on flax but in India only the uredial and telial stages have been found in nature. In Ireland, Pethybridge, Lafferty and Rhynehart (1922, 1923) found all the four stages on fibre flax and successfully infected seedlings of healthy flax in the spring of 1921 with telial material collected in 1919 and subsequently stored indoors. They found that the teliospores could retain their viability for a period of atleast one year and nine months under dry conditions ; under natural conditions they remain inactive during winter and germinate in the following spring. Hart (1926) could not germinate the teliospores of flax rust stored out of doors through the winter in Minnesota before the following June and all her attempts to shorten or break the rest period by exposing the spores to chemicals, as recommended by Thiel and Weiss (1920) for *Puccinia graminis*, or to variations of temperature and moisture,

were unsuccessful. She could not shorten the dormancy period by alternate freezing and thawing either. Allen (1934) found that the teliospores germinated after a much shorter period of rest in the mild Berkeley (California) winter climate. In her experiments, teliospores germinated after sixteen weeks. Collections made by her in the summer of 1931 were viable in June 1932.

In Australia, Waterhouse and Watson (1944) experienced a good deal of trouble on account of sporadic germination of teliospores.

(a) Collections from the plains

Prasada (1940) found that the telia obtained from crops at Cawnpore, Allahabad and Pusa in March, 1939, and stored at 5—7° C. during summer gave nearly 60 per cent germination after 8 months, while those kept in a room in the laboratory at Agra in the plains failed to germinate. This showed that the teliospores are not dead at the time of their formation on the crop in March but are killed on account of exposure to high temperatures after harvest.

Since then, more germination tests have been carried out. Telial material collected from the miniature plots at Simla and from the crop at Pusa in March, 1940, was exposed at Agra to different conditions during summer that year and later tested at Simla for germination, every fortnight. A part of both these collections was retained at Simla and germination was tested simultaneously for comparison. The material was exposed at Agra to the following conditions on May 6, 1940:

(*i*) kept inside a room

(*ii*) under the shade of a tree in a cheese-cloth bag

(*iii*) exposed to direct sun in the open.

Results of these tests are summarized below:—

(*i*) Material kept inside the room at Agra for 9 days showed only 5 per cent germination. After exposure for one month, it lost all viability. Samples received from Agra after 1½ and 2 months also did not show any germination, while teliospores retained at Simla as control gave 60—75 per cent germination in every test. The minimum and maximum temperatures recorded in the room at Agra during the period of exposure, *i.e.*, May 6 to June 3, were 32·5° and 39°C, respectively.

(*ii*) Material kept under the shade of a tree in cheese cloth bags lost all viability after 9 days. Subsequent samples received after one, one and half and two months were dead and no germination was observed. As already stated, the controls retained at Simla gave 60—75 per cent germination in every case. Maximum and minimum daily temperatures recorded in shade at the Agra College Laboratory during May and June 1940, are given in the Appendix. Day after day, the maximum temperature was near or above 43° C during those months.

(*iii*) Material exposed to direct sun at Agra got killed within 9 days, as expected.

It has been stated above that a collection of this rust taken from the crop in the plains in April was received by the writer on June 30. Teliospores from that collection did not germinate at all even after 6 alternate dryings and wettings. Apparently they were killed on account of exposure to the summer heat of the plains.

The effect of exposure to high temperatures on the viability of teliospores was experimentally determined by keeping infected straw at different temperatures

between 30° and 50°C. for 6 to 36 hours. Germination of material exposed to high temperatures and that of control was tested at room temperature at Simla. Whereas the control gave 60—75 per cent germination, material exposed to 33—38°C. for 36 hours showed only 10—15 per cent viability. Hardly one per cent spores were left viable after the same period of exposure at 38—43°C. and no germination was observed after 36 hours' exposure at 43—50°C.

In order to see how exposure to higher temperatures for 12 hours followed by room temperature for 12 hours, alternately, affects viability, material was exposed to different temperatures for 12 hours every day and then taken out in the night; this was repeated for seven days. It was found that material showing 60—75 per cent germination before exposure suffered a gradual decline in viability on every successive day at all the temperatures. At 33°—38°C. only 5 per cent germination was noticed after 7 days. At 38°—43° and 43—50°C. all viability was lost after five and three days, respectively.

Straw bearing telia from the previous year's crop and lying near the fields under natural conditions at the Bichpuri Farm (Agra) was collected towards the end of November, 1941, to see if the spores were viable and capable of producing infection on the new linseed crop. All the spores were, however, found to have been killed.

These results confirm the view expressed by the writer in 1940 that teliospores lose their viability in the plains of India due to exposure to high temperatures after harvest.

(b) **Collections from the hills**

As stated by Collet (1921), linseed is cultivated in this country up to 6,000 ft. in the hills. In the Simla hills it is grown sparingly in the lower altitudes (up to 3,000—4,000 ft.) and collections were made from the crop at Arki (altitude 3,200 ft.) for this study.

Infected straw bearing the telia was collected during the month of May in 1940 and again in 1941 and treated as follows :—

(*i*) Exposed to natural conditions in a cheese cloth bag in the field about one foot above the ground at Arki.

(*ii*) Kept inside a room at Arki.

(*iii*) Exposed to natural conditions in a cheese cloth bag about one foot above the ground in the laboratory at Simla.

(*iv*) Kept inside the room at Simla.

All the four samples were tested, simultaneously, every month for germination at 10°-15°C.

The results are given below :—

(*i*) Material exposed to natural conditions at both the places showed nearly 50 per cent teliospore germination during the first week of June and July. In tests made in the beginning of August only a few teliospores were found to germinate. In September the material did not germinate at all and the teliospores looked empty. Since the temperature was quite congenial and the material stored indoors continued to germinate it appears that the spores germinated *in situ* on account of the presence of free water during the rainy season.

(*ii*) Material stored in the room at a dry place at both the localities was found to retain its viability up to the month of December when further trials were stopped. That stored at Simla gave nearly 40—50 per cent germination throughout this period, while the material kept at Arki showed a slight drop as the period of storage advanced; from 40—50 per cent in June-July to 10—20 per cent in November—December.

Material collected from the miniature plots in the laboratory at Simla gave 60—75 per cent germination throughout the year except during and shortly after the rainy season when 25—33 per cent germination was observed. This can be easily understood because excessive moisture and free water present in nature during the rainy season must have caused most of the teliospores to germinate on the plants.

Tests were made with the teliospores collected from the greenhouse cultures maintained at Simla and 30—85 per cent germination was noticed throughout the year.

Whereas teliospores lose viability in the plains during summer that follows the harvest, it is certain, from what has been stated above, that they are germinable in the hills throughout the summer and, if protected from moisture, right up to the time of the next crop.

(c) Cardinal temperatures for the germination of teliospores

Investigations on the range of temperature suitable for the germination of teliospores of this rust have not yet been carried out. Tests were, therefore, made at Simla, every month from May, 1940, to May, 1942. The results show that the teliospores germinate between 7°—24°C. with optimum in the neighbourhood of 16°—18°C; and that temperature is, as a rule, available throughout the year at Simla.

(d) Dormancy of teliospores

Pethybridge, Lafferty, and Rhynehart (1922) state that teliospores of this rust remain inactive during winter and germinate in the following spring in Ireland. Hart (1926) could not secure their germination before the following June in Minnesota. Hiratsuka (1928) obtained positive results with overwintered teliospores after several unsuccessful attempts. Allen (1934) observed their germination towards the end of May after the mild Californian winter. In all these cases germination was noticed with the onset of warm weather in spring and it was concluded that telispores require a period of rest or maturation. From what has been described by these workers there is nothing to show if germination tests made during winter were carried out under a favourable temperature range.

During the present studies, contrary to observations made by these workers, teliospores produced in the miniature plots or the greenhouse at Simla began to germinate without any rest period or the administration of any special treatment provided the tests were made within 12°—18°C. Within ten days of their formation 10—50 per cent germination was observed.

Teliospores collected in May from crops in the hills and in March—April from places in the plains were also found to germinate between 12°—18°C soon after collection. There was no evidence of a rest period in any collection.

Material stored in the room at Simla and at 5°—10°C remained viable for nearly a year after collection.

THE AECIAL STAGE

Butler (1918) states that only the uredial and telial stages of this rust had been observed in India. The writer (Prasada, 1940) reported the formation of pycnia and æcia as a result of inoculation experiments for the first time in India in that year.

Young leaves of linseed were inoculated with sporidia in the following way :—

A piece of straw containing telial material was soaked in tap water overnight. Next day a portion of the tissue was scraped with a lancet needle on a microscopic slide in a drop of water and teliospores separated from the tissues. The teliospores were then floated on a thin film of water on the slide which was put in a moist chamber at 12°—18°C. The slide was examined every day. Generally on the fourth day, signs of germination were noticeable, when the teliospores were tranferred by means of a sterilized platinum loop to young leaves and stems previously atomized with water and kept inside a glass case. The glass cases were then sprayed after inoculation and the plants were kept covered for three days. The glass cases were sprayed several times during this period to maintain sufficient moisture on the inoculated parts. After three days the plants were transferred to greenhouse tables where they received diffused light. In this way successful infection was obtained between the temperature range 10°—27°C with the production of pycnia in 8 to 9 days and æcia within 15 days.

Inoculations carried out at Simla during the cold weather suggest that temperatures below 10°C are not favourable for the production of æcia although slight germination of teliospores and formation of pycnia may take place even at 7°C. Pycnia so formed may lead to the formation of æcia if suitable temperature (10°—15°C) is available soon after their formation ; otherwise the mycelium may die on account of low temperature.

(a) Germination of æciospores

Hart (1926) observed that æciospores germinate readily in distilled water between 0°—27°C with an optimum at 18°C. No appreciable difference was noticed by the writer in germination tests made in distilled water or tap water. The spores germinated readily between 5° and 27°C and within an hour at 16°—18°C which was found to be the optimum. Exposure for six hours at 43°—50°C and 12 hours at 38°—43°C killed all the aeciospores.

(b) Infection experiments

Plants inoculated with æciospores and kept within the moist chamber for one and half hours did not get infected ; those kept for three hours became slightly infected showing that some of the germ tubes had succeeded in entering the host tissues. Plants kept within the moist chamber for 6, 9 and 12 hours became most heavily infected. In every case where infection took place, the uredial stage was produced.

(c) Longevity of aeciospores in storage

Investigations on the longevity of the æciospores of this rust under different conditions of storage do not appear to have been carried out. Hoerner (1921) reported that æciospores of *Puccinia coronata* from herbarium specimens of *Rhamnus* were viable after a period of 167 days from the date of collection. Aeciospores of *P. impatientis* were found by Mains (1924) to give good germination

after dry storage in a packet at room temperature for 25 days and of *P. triticina* for 5 days. Cotter (1932) states that æciospores of *P. graminis* are capricious in germination and viability.

During these studies æciospores of *M. lini* were kept at four different temperatures in dry sealed glass tubes and germination was tested at the end of every week at 10°—15°C. The results are presented in Table IV and show that the æciospores lose their viability very soon at–5° and 0°C; slight germination was, however, obtained at the end of 4—5 weeks after storage at 7°—10° and 10°—15°C.

TABLE IV

Viability of æciospores under storage at different temperatures. (*Nearly 60 per cent germination was recorded before the material was stored*)

Weeks of Storage.	Percentage germination at			
	–5°C.	0°C.	7°–10°C.	10°–15°C.
1	10	30	50	40
2	T	10	30	20
3	0	T	25–30	10
4	0	0	10–20	T
5	0	0	T	0
6	0	0	0	0

T=trace, *i.e.*, less than one per cent germination

PHYSIOLOGIC SPECIALIZATION

The possibility of the presence of physiologic races within *Melampsora lini* was suggested by Koernicke as early as 1865 when he found that in Prussia *Linum usitatissimum* was often free from rust under conditions in which *L. catharticum* rusted commonly. Arthur (1907), however, did not find any specialized forms. Eriksson (1912) reported two forms of *M. lini*, one occurring on common flax, *Linum usitatissimum*, and the other on *L. catharticum*. Buchheim (1915) demonstrated the presence of several specialized forms as a result of cross inoculations on 12 species of *Linum*. Hart (1925, 1926), found that the urediospores from *L. usitatissimum* did not infect *L. lewisii* and *vice versa* but those of the former heavily infected *L. rigidum*.

The occurrence of physiologic races in Australia was first hinted by McAlpine (1907) when he stated that Russian and Japanese varieties of flax were free from rust whereas the Calcutta variety was heavily rusted. Flor (1935) selected eight differential hosts for isolating physiologic forms of this rust. Vallega (1938) distinguished two physiologic races in Argentina, different from those occurring in U. S. A. He also reported a change of constitution in the indigenous population of the rust as judged by the behaviour of certain varieties such as Ottawa 770 B and Italia-Roma, which were immune in the epidemic season

of 1934, later becoming susceptible. Straib (1939, 1 and 2) differentiated eight physiologic races from urediospore collections from Holland, Sweden and Germany, apparently different from those previously described by Flor. The precise identification cannot, however, be determined because Straib varied the technique employed by Flor and used additional differential hosts. Straib also noticed modifications in the reactions of plants at various stages of growth and inoculated the cotyledonary leaves in the stage of incipient development for the determination of physiologic races. Flor (1940) added three more varieties to his former list of differential hosts thus bringing their number to eleven. An analytical key and reaction of eleven differential hosts to 24 physiologic races were also given. Waterhouse and Watson (1941, 1944) found six new physiologic races of this rust in Australia, not included by Flor in his key of 24 races. Vallega (1944) isolated five races in Argentina, out of which two were new.

In the absence of seed of differential hosts selected by Flor (1940), a preliminary study was made on fifty varieties of linseed and flax with seven rust collections obtained from the Punjab, United Provinces, Bihar and Central Provinces, the chief linseed growing tracts in this country. After collection the cultures were increased on a susceptible variety of linseed in separate double muslin compartments in a greenhouse.

Ten to fifteen plants of each variety were used in small 3-inch pots. Inoculations were made on the seedlings by applying the spores with a small camel hair brush, at the stage when the cotyledonary leaves had fully opened and the first leaves had been formed. The results are presented in Table V.

TABLE V

Reactions of fifty varieties and crosses of Linum usitatissimum *to seven collections of* Melampsora lini

Name of variety	Reaction* produced by collections from						
	Arki 1941 †	Arki 1942	Karnal 1942	Allahabad 1942	Pusa 1942	Nagpur 1942	Seoni 1942
Linseed							
I. P. 12	S	S	S	S	S	S	S
I.P. 124	S	S	S	S	S	S	S
R.R. 3	S	S	S	S	R	R	R
R.R. 9	R	R	R	R	R	SR	SR
R.R. 68	R	R	SR	SR	SR	SR	SR
R.R. 135	R	R	S	R	S	R	R
R.R. 280	SR	SR	SR	S	SR	SR	SR

* Symbols after Flor (1940). The letters signify the following :
I—immune ; R—resistant ; SR—semiresistant ; S—susceptible

† Year of collection of rust

TABLE V—*contd.*

Name of variety	Reactions* produced by collections from						
	Arki 1941†	Arki 1942	Karnal 1942	Allahabad 1942	Pusa 1942	Nagpur 1942	Seoni 1942
Linseed							
R.R. 328	R	R	R	R	R	R	R
O.S.X.	S	S	S	S	S	S	S
E.B. 3	S	S	S	S	S	S	S
F. 55	S	S	S	S	S	S	S
33 B.	S	S	S	S	S	S	S
43 B.	S	S	S	S	S	S	S
C. 483	S	S	S	S	S	S	S
C. 1150	S	S	S	S	S	S	S
C. 1190	S	S	S	S	S	S	S
C. 1206	S	S	S	S	S	S	S
3243	S	S	S	S	S	S	S
3255	S	S	S	S	S	S	S
I.C.R. I	S	S	S	S	S	S	S
I.C.R. II	S	S	S	S	S	S	S
I.C.R. III	S	S	S	S	S	S	S
I.C.R. IV	S	S	S	S	S	S	S
Type 5	S	S	S	S	S	S	S
Type 8	S	S	SR	S	S	S	S
Type 19	S	S	S	S	S	S	S
Type 21	S	S	S	S	SR	S	S
Type 22	S	S	S	S	S	S	S
Type 23	S	S	S	S	S	S	S
Agra local	S	S	S	S	S	S	S

*Symbols after Flor (194). The letters signify the following:
I—immune; R—resistant; SR—semiresistant; S—susceptible
†Year of collection of rust

TABLE V—*concld.*

Name of variety	Reactions* produced by collections from						
	Arki 1941†	Arki 1942	Karnal 1942	Allahabad 1942	Pusa 1942	Nagpur 1942	Seoni 1942
Linseed							
Arki local	S	S	S	S	S	S	S
Karnal local	S	S	S	S	S	S	S
Linseed x Flax hybrid							
No. 370	S	S	S	S	SR	SR	SR
No. 373	S	S	S	S	S	S	S
No. 388	S	S	S	S	S	S	S
No. 395	R	R	R	R	R	R	R
Flax							
Laplata	S	S	S	S	S	S	S
Danubian	S	S	S	S	S	S	S
Baltic	S	S	S	S	S	S	S
Cyprus	S	S	S	S	S	S	S
Egyptian	S	S	S	S	S	S	S
Morocco	S	S	S	S	S	S	S
Fx. 2	SR	SR	SR	R	SR	S	SR
Fx. 8	S	S	S	S	R	R	R
Fx. 9	SR	SR	SR	SR	R	R	R
Fx. 10	SR	SR	S	R	R	R	R
Tall	R	R	S	R	R	R	R
Tall No. 9	S	S	S	S	S	S	S
J.W.S.	R	R	R	R	R	R	R
J.W.S. (C. P.)	R	R	R	R	R	R	R

*Symbols after Flor (194). The letters signify the following:
I—immune; R—resistant; SR—semiresistant; S—susceptible
†Year of collection of rust

On the basis of results presented in Table V, it is possible to identify five physiologic races (provisionally numbered as Simla 1—5) in the rust collections studied, according to the analytical key given below. The reactions produced by single spore cultures of these races on varieties which serve to differentiate between them are recorded in Table VI.

Analytical key on which the identification of Races Simla 1-5 has been based

"Tall".....Susceptible .....*Race* 1
Resistant .....(Races 2, 3, 4, & 5)
"Fx. 8".....Susceptible .....(Races 2 & 3)
"R.R. 280"..Susceptible .....Race 2
Semi-Resistant .....Race 3
"Fx. 8".....Resistant .....(Races 4 & 5)
"R.R. 135"..Susceptible .....Race 4
Resistant .....Race 5

TABLE VI

Reactions produced by races Simla 1-5 on selected varieties of linseed and flax for their preliminary differentiation. (Symbols after Flor, 1940)

Place of Collection	Race	Reactions produced on selected varieties							
		Tall	Fx. 8	R.R. 280	R.R. 135	Fx2	Fx9	J.W.S.	IP 12
Karnal	1	S	S	SR	S	SR	SR	R	S
Allahabad	2	R	S	S	R	R	SR	R	S
Arki	3	R	S	SR	R	SR	SR	R	S
Pusa	4	R	R	SR	S	SR	R	R	S
Nagpur	5	R	R	SR	R	S	R	R	S

GENERAL DISCUSSION

Melampsora lini is an autoecious, full cycle rust. In other countries, such as Argentina, United States, Canada, Ireland and Germany, linseed is cultivated during summer, the telial stage being formed in the fall. The rust is perpetuated by means of the teliospores adhering to the stubble or mixed with the seed. These teliospores germinate and produce pycnia and æcia on the new crop towards spring while successive cycles of uredial stage are completed on diseased plants.

In India linseed is sown in October-November and harvested in March-April. The rust, as a rule, first appears in February. It has been shown that urediospores exposed to 38°—43° C are killed in 24 hours, and it is obvious that they cannot survive the summer heat of the Indo-Gangetic plain or the peninsular India. A culture of this rust could not be maintained at Agra, in the plains, after

the month of April, and even the linseed plants were killed by heat in June. It is apparent, therefore, that the rust cannot oversummer in the uredial stage in the plains on account of excessively high temperature.

The teliospores have been found to be germinable at the time of their formation in March but they lose their viability by subsequent exposure to intense heat of the Indian summer. Teliospores collected in November from old straw of the previous year's crop lying in the fields of Bichpuri Farm at Agra were found to be dead. Material collected from the crop at Karnal in April and sent to Simla in June did not show any viable teliospores. Similarly, teliospores adhering to the seed and stored with it indoors would lose their viability in summer because the telial material kept in a room at Agra in May showed very little germination after 9 days and was completely killed after one month.

All the available evidence, coupled with the fact that the rust breaks out 2-3 months from the date of sowing in the plains, suggests that there is no local source of infection in the plains, from the previous crop.

In the hills, on the other hand, a culture of this rust was maintained in the uredial stage under natural conditions at Simla (altitude 7,200 ft.) for six years and the urediospores remained viable in all the seasons. This shows that the rust can oversummer as well as overwinter in the hills and that, given a congenial host from the time of harvest to the next crop, the disease could be carried over in the uredial stage. Linseed has been grown in small plots at Simla without any difficulty throughout the year along with the rust and it is very likely that a careful search at such places in the hills where linseed is cultivated (according to Collet it is cultivated up to 6,000 ft.) may reveal the presence of infected volunteers during the critical period. In Australia, Waterhouse and Watson (1944) have recorded the widespread natural infection of *Linum marginale*, which is a perennial wild plant. A similar situation might exist in the hills of India and thus enable the rust to oversummer. It may, however, be stated that wild species of *Linum* have not been recorded in India so far.

Apart from the likelihood of the oversummering of this rust in the hills, the teliospores formed there on the crop have been found to be germinable soon after formation and to retain their viability throughout the summer, and, if protected from moisture, till the next crop. Temperature is available throughout the year at Simla for the germination of teliospores, production of sporidia and the infection of linseed plants.

Whether the rust oversummers in the hills on volunteer plants or perpetuates by means of teliospores or both, from the evidence obtained during these studies it appears that the crops in the hills get infected first and then fresh urediospores are blown by wind to the plains causing fresh outbreaks. That would also account for the absence of the æcial stage in the plains.

Preliminary tests made on fifty varieties of linseed and flax with collections from the Punjab, United Provinces, Bihar and Central Provinces indicate the presence of atleast five physiologic races of this rust in India. Since this rust is heterothallic and æcia may be formed in the hills, new races may arise through hybridization.

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SUMMARY

Urediospores of *Melampsora lini* germinate freely between 3°—30°C with optimum near 15°—16°C. All viability was lost when fresh spores were exposed to 38°—43°C for 24 hours.

Temperatures between 13°—21°C appear to be most favourable for the infection of the host and the development of the rust. At this temperature rust appeared within 7 days, the shortest incubation period noticed. The longest incubation period was 18 days at 2°—10°C.

The culture of this rust was continuously maintained in the uredial stage in the greenhouse as well as in miniature plots at Simla, in the hills, for six years.

In the plains, at Agra, on the other hand, cultures could only be maintained during November to April. All attempts to re-establish the rust during the hot weather, *i.e.* May to October, with fresh urediospores sent from Simla, uniformly failed.

Of all the temperatures at which the urediospores were stored, they remained viable longest (18-20 weeks) at 5°—7°C.

In the plains the teliospores are viable at the time of their formation on the crop in March but are killed by very high temperatures prevailing after harvest. They lose their viability completely after exposure to 43°—50° C for 36 hours.

On the other hand, the teliospores are capable of germination soon after formation in the hills and retain their viability throughout the summer and, if protected from moisture, right up to the time of the next crop.

The teliospores germinate between 7°—24°C with optimum near 16°—18°C. Temperature has been found to be generally suitable throughout the year at Simla for the germination of teliospores and production of sporidia and, consequently, for the infection of plants.

The æcial stage has not been found in nature on the crop. Successful infection has, however, been obtained artificially in the greenhouse between 10°—27°C resulting in the formation of pycnia in 8-9 days and æcia within 15 days. Temperatures below 10°C are not favourable for the production of æcia.

The æciospores germinate readily in tap water within an hour under optimum conditions. Germination was obtained between 5°—27°C with optimum at 16°—18°C. Six hours' exposure at 43°—50°C killed all æciospores. Their viability was lost very soon under storage at all the temperatures tested, especially at-5° and 0°C. Some viability was retained for four weeks at 7°—10°C and for three weeks at 10°—15°C.

Five physiologic races could be identified on the basis of the reactions of fifty varieties of linseed and flax to seven rust collections obtained from the Punjab, United Provinces, Central Provinces and Bihar.

The relative importance of different factors that could be responsible for the annual recurrence of this rust in the hills and the plains of India is discussed in detail.

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APPENDIX

Daily minimum and maximum temperatures in degrees Centigrade in shade at Agra College Laboratory during May and June, 1940

Date	May, 1940		June, 1940	
	Minimum	Maximum	Minimum	Maximum
1	29	39	31	43
2	23	40	30	42.5
3	26	40	31	40
4	26	41	24.5	42.5
5	27	41	27	39
6	..	43	28	42.5
7	24	43.5	30	44.5
8	20	42.5	31	46.5
9	23.5	43.5	30	45
10	25.2	43.5	32.5	46.5
11	25.2	45	34	47
12	27.5	45.5	34.5	47
13	29	44.5	33.5	46.5
14	28.5	44.5	33.5	44.5
15	25.2	44.5	32.5	44.5
16	28	45	30	41.5
17	29	43.5	..	..
18	29	44	32.5	45
19	..	43.5	33	44.5
20	27	40.5	27	41.5
21	28	43.5	28	37
22	29.5	46	29	41
23	28	44.5	31	42
24	31	47	32	44.5
25	34.5	44.5	27	42
26	33.5	44.5	30.5	40
27	29	44.5	31	40
28	29.5	40	32	42
29	32	42.5	32	40
30	31.5	44.5	27	36
31	32.5	43.5	..	..

PHYTOPHTHORA SPP. OF POTATOES (SOLANUM TUBEROSUM L.) IN THE SIMLA HILLS

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SEVERAL species of *Phytophthora*, viz., *P. infestans* (Mont.) de Bary, *P. erythroseptica* Pethyb., *P. parasitica* Dast., and *P. drechsleri* Tucker have been isolated from potato plants and tubers. Of these *P. infestans* is, of course, widely distributed but the others are restricted in their distribution. In India only *P. infestans* is so far known to attack potato plants and tubers, and is confined to northern India where it usually occurs at elevations over 4000 feet above the sea level, being common in the Simla, Darjeeling and Shillong hills. In the other mountainous regions of northern India, late-blight, the disease which this fungus causes, is rather sporadic.

In the Nilgiris, in southern India, where three crops of potatoes are raised in a year, late-blight is unknown even though conditions are ideal for its incidence for a major part of the year. Reasons for the entire absence of this disease in those hills deserve, therefore, a detailed study.

In the plains of India late-blight does not, as a rule, occur. Butler (1903) records an outbreak of it in some villages of Bengal and Dastur (1915) in Rangpur (Bengal) and Bhagalpur (Bihar). It was once observed at Pusa (Bihar) and recently it caused a mild epidemic in the Meerut Dt. (U. P.). In 1947 the Potato crop at Patna (Bihar) was badly affected by late-blight and an enquiry revealed that the seed had been brought from Nepal in November 1946. That the incidence of this disease in the plains is intimately associated with the import of seed potatoes at the time they are to be planted, that is, at the beginning of November, from affected areas in the hills, is thus clear. But for these exceptions, the potato crop in the plains is free from late-blight.

In the Simla hills the crop is sown from the end of March to the beginning of May, depending on the elevation of the area where the sowing is done. At Kufri (8000 feet.) where planting is done by the end of March, late-blight has been observed about the middle of July, and at Simla (6800 feet) where the crop is planted in April, a couple of weeks later. Records in the Division of Mycology of the Indian Agricultural Research Institute at New Delhi show that late-blight has been reported from elevations lower than Simla (6800 feet), such as Solan (4900 feet), as early as the middle of May where the crop is sown in February-March; but it is possible that the plants were affected by leaf-rot, which is hereafter described, and had been mistaken for late-blight.

Two new diseases caused by Phytophthora spp.

In the Simla hills, the writer has observed two new diseases of the potato crop caused by species of *Phytophthora*. One of these diseases has been found only on the foliage, and does not appear to affect tubers ; it occurs in the fields between Solan and Simla (4900 to 6800 feet). The second is found at Kufri (8,000 feet) and attacks only the tubers ; the fungus has not so far been observed on the above ground parts. The former disease usually appears sometime in the month of May and June and the latter has been noticed after the middle of July.

Leaf-rot disease found between Solan and Simla

The earliest symptom of leaf-rot is the appearance of a black and water-soaked spot on the leaves, which gradually increases in size as the infection progresses ; the whole leaf ultimately turns black and develops a distinct wet-rot. On the late-blight infected leaf, a brown spot is first manifest which gradually turns black, and if the atmospheric conditions are moist the diseased area on the leaf gets surrounded by a white, downy ring, because of the development of sporangiophores and sporangia ; such a ring is completely absent on leaves affected by the new leaf-rot disease. When the entire plant is completely infected and the rot has advanced, it may not be possible to differentiate this leaf-rot from late-blight, without a microscopic examination. It is not known if the fungus that causes leaf-rot also infects tubers in the field. Potato tubers inoculated on the "eye" or the undamaged skin with the leaf-rot fungus took infection rather slowly ; a dark or blackish discolouration was noticed on the skin about three weeks after the tuber was inoculated ; when the inoculum was placed on the cut surface of the tuber a slight depression developed ; the tissues near about the inoculum turned rusty brown but the flesh remained hard. Twelve potato tubers were inoculated by the following method ; they were first sterilized for over half an hour by keeping them in a solution of corrosive sublimate, 1 in 1,000 ; the solution was drained off and when the tubers were dry a small triangular piece, about one-fourth of an inch long was removed from each tuber by means of a flamed knife; a bit of the inoculum was introduced in the cavity thus made and the triangular piece was replaced in its proper position ; the cuts on the surface of the tuber were covered with hot melted paraffin wax ; these tubers were kept in a refrigerator for a fortnight. There were no external signs of infection ; when the tubers were cut open the flesh was found to be hard and healthy except that a layer of cork was formed on the cut surface of the cavity ; the infection had not spread into the flesh ; aerial mycelium bearing sporangia had developed on the corky surface of the cavity and on the shrunken triangular cut piece ; these cut tubers were placed in sterilized petri dishes with covers and were then kept in the refrigerator ; a film of water was soon formed on the inside of the petri dish cover ; when the tubers were examined after three weeks they were found to be healthy ; the infection had not spread to the flesh ; aerial mycelium and sporangia were seen in the cavity where the inoculum had been originally placed.

Another set of tubers was similarly inoculated with *P. infestans* and kept under identical conditions ; when the tubers were examined after a fortnight the skin was found to be discoloured black in places ; the infection had spread to the flesh; this was evident even to the naked eye by the presence of rust coloured areas on the flesh commencing from the place of the inoculum; the wounded part was not protected by the development of a corky layer ; sporangia were profusely formed on the cut surface of the cavity ; when the cut tubers were incubated in the refrigerator the infection had spread further, and sporangia had developed on the cut surface.

Judging from these inoculation experiments it is probable that even in nature this leaf-rot fungus may not infect the tubers.

In addition to the microscopic differences shown by this leaf-rot fungus, several differences in the morphology of the *Phytophthora* responsible for this disease and of *P. infestans* have been noted. The sporangiophore is branched and does not differ materially from the hyphae, the bulbous swellings seen at intervals on the sporangiophores of *P. infestans*, indicating the places from where the sporangia

have got detached, are entirely absent. The sporangia are much larger than those of *P. infestans*. They are hyaline, pear-shaped or globose with a prominent papilla; at the base of the sporangium there is no remnant of the sporangiophore ; they measure 20·0—62·5μ long and 15·0—47·5μ broad ; (Figs. 7—9); the sporangia germinate conidially or develop zoospores. Chlamydospores have been found in cultures of the fungus on nutrient media ; they are usually round, honey-coloured and thick-walled. They are very variable in size, ranging in diameter on an average from 17·5—42·5μ. They are lateral or intercalar. The oosporic stage develops easily in culture media, antheridia being amphigynous. The oogonial part outside the antheridium is globose, smooth and thin-walled, or encrusted and thick-walled; the thin-walled oogonium is usually hyaline, but may be at times slightly yellowish in colour ; but the oogonium with the encrusted wall may be distinctly yellow in colour or may be hyaline. The diameter of the oogonium is 22·5—45·0μ, usually it lies between 25·0 and 37·5μ; the average of 100 measurements was 31·26μ ; the wall of the oogonium is usually less than 2·5μ thick. The oospore is round, almost completely fills the oogonial cavity and is smooth walled ; the wall is about 2·5μ thick ; the oospore measures 17·5—37·5μ; usually it lies between 22·5—32·5μ ; the average of 100 measurements was 26·3μ; the oospore is usually hyaline but may be tinged slightly yellow (Figs. 10 and 11).

The measurements of the sex organs of this fungus are very much like those of *Phytophthora parasitica* Dastur, the cause of damping off of seedlings of castor, *Ricinus communis*. A few differences in the morphology of these two fungi are, however, brought out when they are grown on Leonian's agar ; the potato fungus develops globose or pear-shaped sporangia which do not produce zoospores but germinate conidially ; chlamydospores and sex organs are not found ; the hyphae put forth short thick branches. *Phytophthora parasitica* forms pear-shaped or irregularly shaped sporangia which germinate as a rule by the formation of zoospores, and at times by the formation of germ-tubes ; chlamydospores are formed in abundance when grown on this agar, though the sex organs are lacking. The hyphae are long and not much branched.

Variations in tolerance to malachite green by the potato *Phytophthora* and *P. parasitica* are also distinct. The former grows in Leonian's solution to which malachite green has been added in the proportion of one to two million, one to four million, one to eight million, or one to sixteen million parts of the dye, forming a flocose growth, whereas the latter does not grow in the nutrient medium to which the dye has been added in the proportion of one to two million ; but at lower concentrations, *viz.*, one to four million, one to eight million and one to sixteen million, the fungus forms a cottony ball-like growth.

Even in their parasitism the two fungi show differences. When leaves of castor seedlings were inoculated with the two fungi, the potato *Phytophthora* produced black lesions not unlike those caused by it on its host, whereas *P. parasitica* caused brownish dry spots. The former produced ample aerial mycelium on the leaves, while the latter did not develop it.

Potato plants inoculated with the leaf-rot fungus developed typical wet-rot symptoms on the leaves, but those inoculated with the castor *Phytophthora* produced only slight necroctic areas where the inoculum was placed on the leaves.

Small plants of tomato (*Lycopersicon esculentum*) were successfully inoculated with the castor *Phytophthora* and the Simla potato *Phytophthora;* but the inoculated

fruits showed some difference, the castor *Phytophthora* produced only a slight discolouration at the place where the inoculum was placed, there was no aerial mycelium on the discoloured spot; whereas the Simla *Phytophthora* produced a distinct wet-rot in five days after inoculation ; there were on the circular diseased areas small raised white coloured clusters of the mycelium ; there was no fructification. The fruits selected for this experiment were about two-thirds ripe ; the inoculation was through a small cut on the skin.

Apple fruits were similarly inoculated through small cuts in the skin. In a week's time the fruits inoculated with the Simla *Phytophthora* showed only a small rotting area round the inoculum, but the fruit inoculated with the castor fungus showed more prominent signs of infection ; the infection had travelled deeper in the flesh than in the case of the fruit inoculated with the other *Phytophthora*.

It is seen that the *Phytophthora* causing leaf-rot of potato plants at Simla and lower altitudes is not wholly identical with *P. parasitica*. But Tucker (1931) has established that different isolations of the same species of *Phytophthora* have considerable variations in pathogenicity. These variations have been particularly shown in the numerous isolations of *P. parasitica*. As no morphological characteristic is found by which the Simla potato *Phytophthora* can be distinguished from the various isolations of *P. parasitica*, I am inclined to consider the isolation from potato leaves at Simla to be *P. parasitica*, *P. parasitica* has, of course, been isolated from rotted potato tubers by Tucker (1933), but there are no previous records of its having attacked the leaves and causing the disease described here.

Phytophthora species causing tuber-rot at Kufri

Tubers from plants that were severely attacked by late-blight were first collected at Kufri in July 1945 and then in 1946. Some of them showed a dark discolouration on the skin, not unlike tubers affected by late-blight. On cutting open the tubers, the diseased part of the flesh was found to be discoloured ; the discolouration varied from light brown to rusty brown to black ; such discolouration was, however, deeper than in the tubers infected by *Phytophthora infestans*. The cut surface did not turn pink on exposure to air, as in the case of tubers affected by *P. erythroseptica*. The mycelium was inter-and intra-cellular but haustoria were not seen.

It is not possible to say if this fungus attacks also the foliage, as the plants from which the diseased tubers were collected in 1945 and 1946 were badly infected by *P. infestans;* practically every leaf showed typical late-blight infection ; there was a profuse development of the characteristic sporangiophores and sporangia.

Cultures from diseased tubers yielded a fungus entirely different from *P. infestans*. On oat agar, the growth was profuse, cottony and white, not unlike that of *Phytophthora parasitica ; P. infestans* produces on this and other media very scanty growth, and only slight aerial mycelium. Another prominent characteristic of the new isolation is that it produces only sex organs and that too in great abundance, sporangia not having been seen either on the usual nutrient media or on diseased tubers. On the other hand *P. infestans* generally produces only sporangia with typical sporangiophores in nutrient media, oospore formation being rare, and on diseased tubers and leaves.

The antheridia of both these fungi are amphigynous and hyaline The oogonia of the Kufri isolation which develop on nutrient media and on the surface of inoculated potato leaves and tubers are hyaline, smooth and thin walled. But when the inoculum is placed on the cut surface of the tuber the sexual spores developing on the mycelium growing on the surface have coloured oogonia; the oogonial wall

is smooth, thick, yellowish brown or honey-coloured ; the colour may be so deep that the enclosed oospore may not be visible ; the wall is not brittle. The wall of the oogonia of *P. infestans* is thick, reddish brown in colour, very often encrusted and distinctly brittle. The oogonia of the Kufri *Phytophthora*, both coloured and hyaline, measure 19·0—50·0μ in diameter; the mean of 100 measurements being 35·2μ ; those of *P. infestans* measure 31·0—46·0μ, the average being 38μ. The oospores of the Kufri *Phytophthora* are round and hyaline and almost fill the oogonial cavity, the wall is not more than 2·5μ in thickness ; they measure 15·0—45·0μ in diameter, the average of 100 measurements being 30·9μ ; the oospores of the late-blight fungus are round, thick walled, smooth and hyaline ; they measure 28.0—34·0μ the average being 30μ; the thickness of the oospore wall measures 2.0—3·0μ. As already stated, both in nutrient media and in nature sporangia have not been observed but their formation has been induced in ant cultures. Ants for this purpose are sterilized in water in test tubes and inoculated with the fungus. Only mycelial growth is produced ; but when these cultures are placed in watch glasses with only a limited quantity of water, sporangia develop after some time. It has been noted that if the mycelium is submerged in water sporulation is inhibited ; but when it is only partly submerged sporangial development takes place. These sporangia are entirely unlike those of *P. infestans* (Figs. 1—3), they are not pear-shaped or globose or round but slipper-shaped, their apical end is almost as broad as the base and very slightly raised so that the papilla is not very prominent. Rosenburg (1917) considers the degree of the developmet of the papilla of the sporangium to be constant and a distinguishing character for identification of the species ; the papillae of the Kufri *Phytophthora* are practically as broad as the base of the sporangia ; they are very slightly raised and cannot be easily distinguished from the curvature of the sporangial wall as in the case of *P. syrangae* Kleb. and *P. eythroseptica*. The sporangia measure 29·0—50·0μ in length and 22·0—36.0μ in breadth, the papillae being 8.5—13.0×2·5—5.0μ. It will be noted that these measurements indicate that the sporangia are much larger than those of *P. infestans*. Their germination is by the formation of germ tubes, zoospore formation not having been observed. The hyphae in ant cultures from peculiar intercalar swellings (Fig. 4).

Potato tubers inoculated with this fungus readily took the infection, which could be seen by the discolouration of the skin ; a month after the inoculation tufts of white fungal growth were visible pushing out through lenticles and "eyes"; there was no development of sporangia on these hyphae, but hyaline oogonia and oospores were produced. They were identical with those found on nutrient media. When the cut surface was inoculated there was a slight depression at the place the inoculum was placed and the tissues had turned rusty-brown ; the discoloured area spread; the older affected area became blackish in colour ; and the rusty-brown discolouration was on the outside of the old diseased part; the aerial growth wass canty; the mycelium was generally appressed to the surface ; there was no development of sporangia, but sexual spores were formed on the surface ; the oognial wall was smooth, thick and almost opaque; the wall was brown or honey-coloured like that of the oogonia of *P. infestans ;* but there were no encrustations as those found in the late-blight fungus. When the tuber was cut the infection was found to have penetrated deep in the tissues. Cut tubers infected with *P. infestans* produced aerial growth on which sporangia had developed but not oospores ; there was a slight brown discolouration of the flesh just below the skin ; the infected area was not as deep as in the case of the tuber-rot fungus infection. The flesh of the inoculated tubers remained hard; wet—rot was produced only when secondary organisms invaded the tissues.

The method described on a previous page for inoculating tubers with the leaf-rot *Phytopthora* and *P. infestans* was also used for inoculating another set of tubers with the tuber-rot *Phytophthora;* in a week's time the skin of the inoculated tubers was discoloured black in patches like those of the tubers inoculated with *P. infestans ;* on cutting open the tubers the infection was seen to have spread deeper in the flesh ; the hyphae were both inter and intra-cellular; where inter-cellular cavities were large there was a collection of strands of hyphae; there was no cork formation on the cut surfaces of the cavity ; honey-coloured or yellow-coloured oospores had developed on the outside of cut surfaces of the cavity ; when the cut tubers were incubated in the refrigerator there was no aerial development of the mycelium and no formation of the sex organs inside the tissues; the whole of the flesh was infected with hyphae.

Potato plants inoculated with this fungus did not take the infection readily. At the place where the leaf was inoculated there was a small water-soaked lesion four days after inoculation ; there was later a general wet-rot of infected leaves; this may be due to the plant being kept long covered under a bell jar. The hyphae on the lesion were appressed to the leaf surface; there was no aerial sporangipherous hyphal development as in the case of *P. infestans ;* oogonia and oospores were formed on the mycelium on the leaf surface which were hyaline in colour. Castor seedlings inoculated with *P. infestans* and the tuber rot *Phytophthora* failed to take the infection.

The tuber-rot fungus is considered to be a new species of *Phytophthora* and is named *P. himalayensis* Dast.

Phytophthora himalayensis Dastur sp. Nov.

Causing black discolouration on the skin of the tubers ; diseased tissues light brown to rusty brown not turning pink on exposure to air ; infection deeper in the tuber tissues than in *Phytophthora infestans* mycelium inter-and intracellular ; haustoria lacking ; sporangia not seen on host ; in cultures produced only on sterilized ants in water, 29.0—50. $\times$ 22.0—36.0μ ; papilla very broad and very slightly raised, 8.5—13.0$\times$2.5—5.0μ ; sporangia germinating conidially ; zoospores not developed ; special sporangiophores absent; chlamydospores on host or in nutrient media absent ; profuse development of sexual spores in nutrient media; formed also on host ; sexual spores on nutrient media and host hyaline in colour but on cut surface of potato tuber brown or honey-coloured ; antheridia amphigynous ; oogonia smooth and thin-walled, globular or globoid, 19.0—50.0μ in diameter ; the average of 100 measurements being 35.26μ ; antheridia and oogonia borne on the same hypha or on different hyphae ; oospores globular, smooth and not thick-walled, rarely tinged yellow, almost filling the oogonial cavity, 15.0—45.0μ, the average of 100 measurements being 31.0μ.

Hab. in tubers of *Solanum tuberosum* Linn. Kufri, 8,000 ft. (Simla Hills), India. July 1945 and 1946, leg J. F. Dastur.

Type specimens and cultures are deposited at Herb. Crypt. Ind. Orient,. I.A.R.I., New Delhi, India, and the Imperial Mycological Institute, Kew ; specimens have

also been deposited at the New York Botanic Garden, Farlow Herbarium of the Harvard University and the U. S. Department of Agriculture.

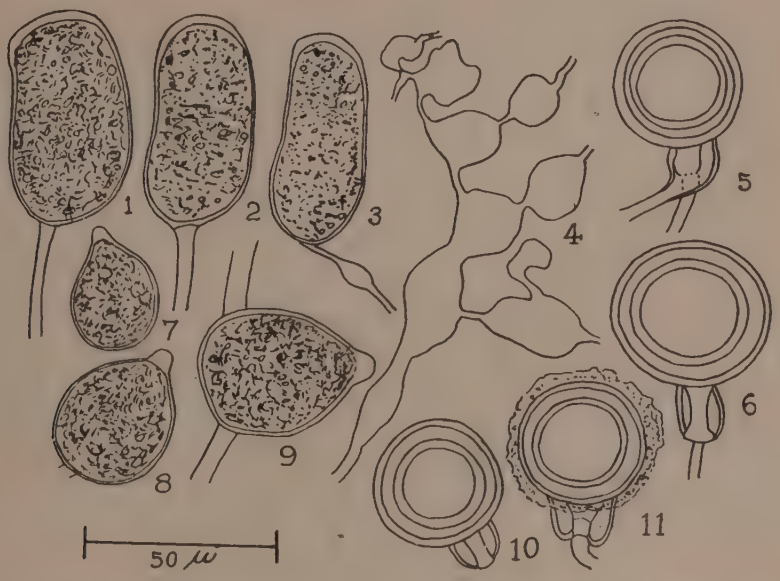

Fig. 1 to 4. Sporangia and mycelium of **Phytophthora himalayensis** grown on ant cultures

Fig. 5 & 6. Oospores of **Phytophthora himalayensis**

Fig. 7 to 11: Sporangia and oospores of **Phytophthora parasitica**

Discolorationes in epidermide tuberum nigrae, in partibus infectis inferioribus pallide brunneae and rubiginoso-brunneae, post ad aerem patefactae colorem ad roseum haud mutantes; quam illae a *Phytophthorae infestantis* productae profufundiores. *Mycelium* ex *hyphis* inter-et intra-cellularibus compositum. *Sporangiophora* specialia nulla. *Sporangia* in planta hospite non visa, sed in culturis ad formicas in aquo sterilisatas evoluta; 29.0—50.0×22.0—36.0μ, papilla latissima et inconspicue eminenti, 8.5—13.0×2.5—5.0μ ornata; zoosporis carentia, et ita ut conidia germinantia. *Chlamydosporae* nullae, et in planta hospite et in culturis absentes. *Sporae sexuales* in medio nutritivo profuse—, etiam in planta hospite nonnihil-, productae, hyalinae, sed in superficie incisa tuberis solanacei brunneae vel melleae; et antheridia et oogonia hyphis vel singulis vel diversis insidentia. Antheridia amphigyna. *Oogonia* pariete tenui praedita, laevia globosa vel globoidea, 19.0—50.0μ diam., (post 100 mensurationes medietas erat 35.26μ.) *Oosporae* globosae, laeves, pariete haud crasso praeditae, colore luteo raro aspersae, cavitatem oogonialem fere omnino implentes, 15.0—45.0μ diam.: (post 100 mensurationes medietas erat 31.0μ).

Hab. in tuberibus *Solani tuberosi* Linn.: Kufri, Simla Hills, Indiae Julio 1945 et 1946. [J. F. Dastur]. Specimenia typi in Herb. Crypt. Ind. Or., I.A.R.I., New Delhi, India, et in Herb. I.M.I., Kew, posita.

SUMMARY

The potato crop in the Simla Hills every year becomes badly damaged by late-blight, *Phytophthora infestans*. Besides this disease, two new diseases caused by species of *Phytophthora* have been found in recent years on this host. One of these diseases, the leaf-rot disease, has so far been found to occur at Simla and at lower altitudes ; its incidence is almost a month earlier than that of late-blight. It is confined to leaves and causes a wet-rot of the affected foliage ; in the early stages of infection the new disease can be readily distinguished from late-blight. As far as the measurements of the asexual and sexual spores are concerned they are not much different from those of *P. parasitica;* but there are certain differences in the physiology and pathogenicity of the two fungi. However, they are not considered to be enough to justify the making of a new species or variety The other disease is confined to the tubers and is therefore named tuber-rot disease. It is found at altitudes higher than that of Simla, about 8000 feet, and infects the potato crop almost at the same time as late-blight. The tubers affected by this new *Phytophthora* externally look like those attacked by *P. infestans ;* however, when the tuber is cut open it shows symptoms different from those attacked by late blight. The fungus isolated from these diseased tubers forms on nutrient media and on inoculated tubers only amphigynous sexual spores, and that too in large numbers. Sporangia and chlamydospores are not formed. The sexual spores are characteristically different from those of *P. infestans.* Sporangia can only be formed on ant cultures and they are different from those of most of the known species of *Phytophthora.* This tuber rot fungus is considered to be new to science and is named *P. himalayensis.*

ACKNOWLEDGMENT

My thanks are due to my colleague, Dr. B. B. Mundkur, for reading the manuscript and for his valuable suggestions, and to Rev.H. Santapau for translating the description of *Phytophthora himalayensis* into latin.

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WILT DISEASE OF PYRETHRUM

By T. S. RAMAKRISHNAN and C. K. SOUMINI

(Accepted for publication March 21, 1948)

THE cultivation of pyrethrum (*Chrysanthemum cinerariafolium* Vis.) in the Madras province received an impetus during the World War II (1939-1946) to meet the requirements of the army in their eastern campaign. Large areas of grassy downs and scrub-jungles were cleared on the upper elevations of the Nilgiri and the Palni hills and pyrethrum was planted. With this extension of cultivation, incidence of certain diseases became noticeable. In the areas at Berijem, near Kodaikanal (Palni hills), it was found that a number of plants exhibited progressive drying of the leaves and shoots, especially during the dry months. Some of these plants put out fresh growths from the side shoots, or suckers, during the rainy seasons but these also succumbed in course of time and the entire plants slowly dried up. It was also noticed that the plants growing on the steeper slopes were more severely affected than those in level areas. The soil from the base of the plants on the slopes gets easily eroded during the rains and some of the roots become exposed. During the dry season each plant appeared as though it had been propped up on a mound of earth. The exposure of the roots may contribute to the gradual drying up of the plants. However, in several of the affected plants the roots were found to be dead and a species of *Fusarium* was isolated on several occasions from such roots. Further examination of diseased plants in the initial stages showing incipient wilting of the leaves, resulted in the isolation of three fungi from the roots *viz.* *Phytophthora* sp., *Rhizoctonia* sp. and *Fusarium* sp. These three fungi were brought into pure culture and their pathogenicity on the host plant was tested. The results of these investigations are recorded in this paper.

Review of previous work

Curzi (1933) records a disease of pyrethrum caused by *Fusarium javanicum* Koorders on the island of Cherso. Ikata (1928) has recorded a wilt and decay of pyrethrum in Japan caused by *Sclerotinia* sp., *Sclerotium rolfsii*, and *Fusarium* sp. New plantings of pyrethrum in Belgian Congo are reported to have been damaged by a species of *Rhizoctonia*. In Colorado a crown rot of pyrethrum (*C. coccineum* Sims.) is attributed to *Phytophthora* sp. Damping off of pyrethrum caused by a species of *Pythium* has also been recorded from this area. In Java, Van der Goot (1935) observed pyrethrum plants severely injured during the rainy season by *Sclerotium rolfsii*. Damping off of pyrethrum seedlings has also been observed in the nurseries on the Nilgiris. *Pythium* sp. was found to be responsible for this disease.

Materials and methods

The isolates of *Phytophthora*, *Rhizoctonia*, and *Fusarium* obtained from the diseased plants from Kodaikanal were purified by transfer of single hyphal tips. They were grown on a number of common agar media at laboratory temperature (26-30°C). Inoculations were carried out on plants grown in pots and kept inside glass cages or covered over with bell jars to give a humid environment.

I. PHYTOPHTHORA CAMBIVORA (Petri) Buis.

This isolate readily grew on oat agar producing white 'cumulus' like aerial growth. Luxuriant aerial growth was evident on potato-dextrose and corn meal agar media forming indistinct zones. On french bean agar there was less of aerial mycelium but zonation was distinct. Reproductive bodies were absent on solid media, but irregularly swollen or rounded vesicular bodies were sometimes developed by the submerged hyphae. In the aerial growth a white powdery dotted appearance was visible on some media *e.g.* french bean agar. These dots were found to be made up of clusters of short, curved hyphal branches.

When bits of culture were placed in watch glasses containing tap water or soil leachate (Mehrlich, 1936) sporangial formation was evident within 24 hours. The sporangia were produced terminally on slender stalks of varying lengths, and much finer than the normal hyphae. The sporangium is obpyriform with a broad apex and non-papillate. It measures 43.0 x 20·5 μ (22.0-71.0 x 11.0–32.5 μ) In the initial stages, however, it is often round, the obpyriform shape being attained in the later stages. Germination both by zoosporangial and conidial methods was noticed. The zoospores varied in number according to the size of the sporangia. Sympodial growth of sporangiophores by the formation of branches from below the older sporangium was common. Proliferation through a germinated or empty sporangium was however absent. Oogonia and oospores were never formed by this isolate throughout the period of two years when the organism was under study. It was grown on carrot agar, onion agar, sterilised carrot roots and tomato stems besides the media already mentioned, but in no case were the sexual bodies noticed.

Fig. 1. Sporangia of *Phytophthora cambivora* (x 400)

Pathogenicity : Inoculations were carried out with the pure culture of the isolate on pyrethrum seedlings and older plants to find out its parasitic ability. The following results were obtained :—

TABLE I

The results of inoculations with Phytophthora cambivora

Host	Age of the plant	Method of inoculation	Number inoculated	Number infected	Results
1. Pyrethrum seedlings	2 months old	Placed in Buchner tubes.	12	12	All the seedlings rotted in the course of the week
2. Pyrethrum young plants	3 months old	Grown in pots ..	6	6	Do
3. Pyrethrum older plants	6 months old	Grown in pots inoculated in the centre of the clump.	6	6	Infection proceeded from the centre outwards and involved the whole plant in the course of 10 days
4. *Chrysanthemum* sp.	Young plant	Grown in pots inoculated at base	6	..	No infection
5. Potato tubers	..	Wound infection	6	6	Rotting was evident in the course of 4 days

Suitable controls were kept in each case and they always remained healthy. Inoculations on pyrethrum plants were made at the base of the plant at ground level.

The results recorded in the above table show that the isolate was parasitic both on pyrethrum seedlings and grown up plants, under humid conditions. The organism was always re-isolated from the infected plants. The hyphae were noted in the tissue of the stem and the roots.

Identity : The non-papillate nature of the sporangium formed in liquid media and the absence of reproductive bodies in solid media suggest the affinity of this isolate to *Phytophthora cambivora* (Petri) Buis. and *Phytophthora cinnamomi* Rands. Oogonia had never been found ; neither was proliferation of sporangia evident. The isolate was able to cause rotting of wounded potato tubers. These characters suggest relationship to *Phytophthora cinnamomi*. But the aerial growth was always white and on solid media the isolate was almost sterile, without any reproductive bodies.

It exhibited characters intermediate between those of *P. cinnamomi* and *P. cambivora*. But for the capacity of the isolate to infect potato tubers it could be readily identified as *Phytophthora cambivora*. *Phytophthora cinnamomi* is stated to assume slightly brownish colour in the aerial mycelium, owing to the formation and profuse development of vesicles. The isolate under study produced always a pure white aerial growth and the formation of vesicles was not profuse on solid media. Therefore, in spite of the capacity of the isolate to infect potato tubers, it is identified as *Phytophthora cambivora*. Mehrlich (1936) has questioned the validity of separating *P. cambivora* from *P. cinnamomi* on the basis of differential susceptibility of potato tubers to infection as attempted by Tucker (1931). On the other hand he is of opinion that the two should be combined into one species. *P. cambivora* is in any case the older of the two names.

II. RHIZOCTONIA SOLANI Kühn

The isolate produced good growth on potato dextrose agar and oat agar. In the initial stages the colour was whitish but later it turned into various shades of brown. The surface of the petri-dishes (10 cms.) was covered in 4 days with radiating stringy hyphae. Along the margin dark brown masses sometimes developed and irregular plates were formed. The hyphae extended to the inner surface of the upper dishes producing a mealy appearance. Similar growth was visible in tube cultures on the sides of the glass. Sclerotial formation was meagre. Few sclerotia developed varying from buff to dark brown colour. They were round and separate or lumped together into irregular masses. Individual sclerotia 0.5 to 1.5 mm. in diameter were often suspended in the loose hyphal mass filling the test tubes. The mealy growth was made of clusters of short repeatedly branched barrel-shaped cells resembling those formed by *Rhizoctonia solani* (Kotila, 1947). The clusters of branches easily broke up into one-celled or two to many-celled portions. Some of the cells were glistening, being completely filled with protoplasm while others were empty. The former germinated when placed in water sending out germ tubes from the apices or from the septa through the connected empty cells. The hyphae may attain a thickness up to 11 μ. The branches arose at right angles or at lesser angles. A septum was evident in the branch a short distance above the place of origin. Clamp connections were rare.

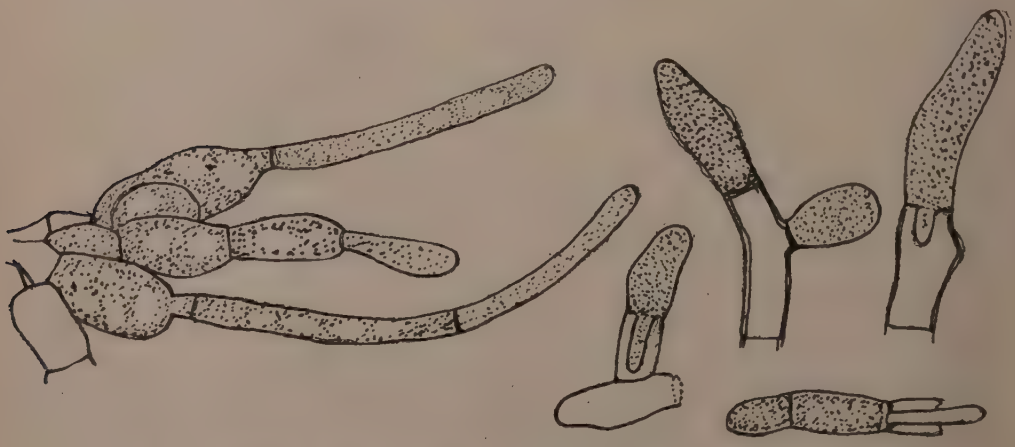

Fig. 2. *Rhizoctonia Solani*, clusters of short cells and their germination (x 400).

TEXT FIG. 2

The growth of the fungus was less profuse on french bean, onion or carrot agar. A thin loose mycelial growth developed on sterilised tissues of carrot and stems of cotton and tomato. Basidia and basidiospores were not formed.

Pathogenicity.

The isolate was inoculated on various host plants to find out its pathogenicity. The results are given below:

TABLE II

The results of inoculation with Rhizoctonia solani

Host	Part inoculated	Number inoculated	Number infected	Remarks
Pyrethrum	Base of seedling 2 months old	6	6	Seedling killed in 5 days
,,	Base of plant 3 months old	6	6	Plants killed in 8 days
,,	Base of plants 6 months old	6	6	Plants killed in 10 days
Chrysanthemum sp.	Leaves of grown up plants.	8	8	Spots developed in 3 days and extended over the whole leaf causing blight.
Cotton (*Gossypium hirsutum*)	Leaves	10	10	Water soaked brown spots formed in 4 days which caused shedding of leaves
Maize	Collar of seedlings	10	10	Seedlings killed in 8 days
Brinjal	Leaves	10	10	Spots formed on the leaves and spread over the major portion of the leaves

Controls were kept in each case and these remained healthy.

The isolate was pathogenic to pyrethrum and also several other host plants. It was reisolated from the infected plants.

Identity

The isolate is pathogenic on pyrethrum and from its morphological characters can be identified as *Rhizoctonia solani* Kühn. Owing to the absence of basidia or basidiospores it has to be kept for the present in the genus *Rhizoctonia* though the isolate exhibits features noticed in cultures of *Pellicularia* (*Corticium*).

III. FUSARIUM sp.

This isolate produces a white mealy growth on oat agar. Before taking up a detailed study of the isolate, its pathogenic ability was investigated by carrying out infection experiments on pyrethrum. The isolate was inoculated on seedlings and older plants. Not a single instance of positive infection was obtained. Consequently, further study of the isolate was abandoned. The results indicated that this isolate was not really pathogenic on pyrethrum, though it had been isolated on several occasions from diseased or dead pyrethrum plants.

DISCUSSION

Three fungi, *viz. Phytophthora cambivora, Rhizoctonia solani* and *Fusarium* sp., are associated with the wilt disease of pyrethrum in the Palnis. Infection studies have shown that only the first two fungi can infect healthy plants and cause a wilt disease. The third fungus though associated with the dead plants is only a saprophyte which follows in the wake of the parasite. The real causal agents can be easily isolated only from plants in the initial stages of the disease. Both *Phytophthora cambivora* and *Rhizoctonia solani* are soil inhabitants and become active when conditions are favourable. Both of them infect the roots and the collar region of the plants. The affected regions are later overrun by *Fusarium* so that the parasites are not easily made out in the final stages of the disease. Such saprophytic growth of *Fusarium* in the wake of infection by *Phytophthora* has been noticed in other diseases like fruit-rot of arecanuts, bud-rot of coconut palms, wilt and foot-rot of betel vines etc.

Soil conditions influence the relative incidence of the disease. The disease is more on steep slopes which are liable to heavy wash and erosion. It is believed that the prevention of erosion will help in reducing the incidence of this disease.

We are grateful to the Cotton Specialist, Coimbatore, for kindly supplying the plants of pyrethrum for the infection experiments.

SUMMARY

A wilt disease of pyrethrum was prevalent in the plantations on the Palnis (Madras Province). Three fungi, *Phytophthora cambivora, Rhizoctonia solani* and *Fusarium* sp. were isolated from the diseased plants. The two former were found to be pathogenic on pyrethrum while the third fungus (*Fusarium* sp.) did not infect healthy plants. The cultural characters and the results of infection experiments of the two fungi are described.

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HELMINTHOSPORIUM DISEASE OF RICE

I. NATURE AND EXTENT OF DAMAGE CAUSED BY THE DISEASE

By S. Y. PADMANABHAN, K. R. ROY CHOWDHRY AND D. GANGULY

(Accepted for publication May 20, 1948)

Helminthosporium oryzae Breda de Haan (*Ophiobolus miyabeanus* Ito et Kuribiyashi) causes the disease of rice known in literature as "Helminthosporiose," "Helminthosporium disease," "Brown-spot" or as "Sesame eye-spot". The crop is attacked by the fungus in all stages of its growth. Thus the fungus infects the germinating seedlings, the growing and mature plants, the inflorescence and the grains. Normally the damage caused by the pathogen is not very serious but under certain conditions, widespread epiphytotics break out and immense losses are caused.

The symptoms of the disease have been described by Sundararaman (1922) and by Mitra (1931) in India. There is a difference, however, in the symptoms associated with the mild type of infection when no serious losses are caused and those that characterise an epiphytotic outbreak. In the former case, small brown spots which are 2-8 x 0.5 mm. in size appear on the leaves and ears. The spots are few in number and their presence on the plants is noticeable only when closely observed. On the other hand, when the disease appears as an epiphytotic the spots are many and run together, the plants are stunted, sometimes the entire leaves wither and whole fields present a highly characteristic, burnt and scorched appearance.

THE CAUSAL ORGANISM

Sundararaman (1922) first reported the fungus in India. On the basis of that description, Mitra (1931) identified it as *H. oryzae*. The fungus has been reported from most of the rice growing countries and though the descriptions given by the various authors agree with one another regarding gross morphology, a wide variation in spore size and septation has been recorded.

TABLE I

Comparison of the conidial measurements of H. oryzæ *as reported by different authors*

Name of author	Size of conidiophores	Size of conidia	Number of septa
Breda de Haan ..	....	90 x 16 μ	....
Hori	100-330 x 6-8 μ	84-140 x 16-22 μ	6-11
Sundararaman ..	70-175 x 5.6-7 μ	45-106 x 14-17 μ	5-10
Drecshler	150-600 x 4-8 μ	35-170 x 11-17 μ	1-12

TABLE I—*concld.*

Name of author	Size of conidiophores	Size of conidia	Number of septa
Hiskado			
on host	68.8-550 x 5-15 μ	23-125 x 11-28 μ	1-12
on media	43-533 x 5-15 μ	17-123 x 10-28 μ	
Mitra	350 μ	30-172 x 10-23 μ (88 x 17)	2-16
Wei			
on host	99-345 x 5-11 μ	24-122 x 7-20 μ	6-7
on media	112-145 x 5-10 μ	49-142 x 8.5-21 μ	3-11
Present Investigation	44-210 x 5-7.5 μ	16-113 x 7-21 μ	2-12

The conidiophores which emerge through the stoma or directly through the epidermal cells in the leaves, arise singly or in fascicles of three or four. They are generally 7-14 septate with a swollen basal cell and with geniculations at the apical end. The conidiophores are 44-210 μ long and 5-7.5 μ broad.

The conidia are, as a rule, curved sometimes straight, or curved to one side, or rarely sigmoid; they are stoutest at the middle or a little below and taper into blunt ends; when the distal end is curved it may be somewhat pointed. The spore walls are thick but in the terminal cells they are generally thinner; these terminal cells are frequently hyaline and may some times be filled with granular contents.

The conidia are 3-12 septate, 7-9 being most common. They vary in size from 16.0-113 x7-2 μ. The percentage occurrence of the different septate spores, their respective mean sizes, and range in length and breadth are presented in Table II. Twenty-five samples were studied representing collections from different localities of Bengal. Spores were obtained from leaf, leaf sheaths and ears of diseased plants.

TABLE II

Showing the occurrence of septate conidia, mean length and breadth, and range in size of the conidia in μ *of* H. oryzae *produced on the host*

Number of septa in conidia	Percentage occurrence	Mean length and breadth in microns	Range in length and breadth in microns
3	rare*	16.0 x 7.0	....
4	rare*	36.0 x 7.0	....
5	3	43.0 x 12.0	35.0-49.5 x 9.0-16.0
6	9	47.5 x 14.0	35.0-77.0 x 10.0-18-.0
7	25	58.0 x 14.0	35.0-83.0 x 10.0-18.0
8	27	65.0 x 14.5	43.0-92.0 x 11.0-20.0
9	21	73.0 x 16.0	45.0-94.0 x 11.0-20.5
10	12	79.0 x 17.0	58.0-103.0 x 13.0-22.0
11	3	79.0 x 18.0	62.0-103.0 x 13.5-22.0
12	rare*	104.0 x 19.0	....

* Below one percent

MATERIALS AND METHODS

The nature and extent of damage caused by the disease in rice was experimentally investigated. Experiments were conducted to find (i) the loss in the weight of grains affected by the disease, (ii) loss in germination of seeds under field and laboratory conditions, (iii) loss in yield resulting from sowing diseased seed and (iv) loss in yield associated with different degrees of infection on the crop. For the laboratory tests on loss in weight and loss of germination, seeds collected from fields, as well as seed samples from departmental stores were used. The seeds were separated into "spotted" and "healthy or unspotted" seeds. Other fungi, besides *Helminthosporium oryzae*, were associated with the spots (*e.g. Trichoconis caudata* (Apps. and Str.) Clements, and *Curvularia lunata*, (Wakker) Boedijn. No attempt was made to separate the individual grains with *Helminthosporium* spots alone. But each sample was examined and *Helminthosporium oryzae* identified in the grains before inclusion in the tests.

For the field experiments, seeds of the variety "Latisail" were obtained from Mayanagari in North Bengal. This variety was severely attacked by the *Helminthosporium* disease in 1943-44. The grains were severely spotted by the fungus.

After harvest, it was found that 56% of the grains had *Helminthosporium* spots. Therefore this variety from Mayanagari was selected as a standard in the experiments described below.

(i) Nature of damage to seedlings

During the three years, 1943-46, only a few isolated instances of seedling injury have been observed under field conditions. In moist chamber trials under laboratory conditions, seedling injury was commonly observed. In the field as well as in the laboratory two types of attack have been noticed. A type of post emergence damping-off was met with, following root infection in which profuse gray mycelium of the pathogen overgrew the dying seedlings under laboratory conditions in the field ; the initial infection was followed by the collapse of the seedlings. Typical "collar rot" symptoms constituted the second type of infection. A brown ring appeared beneath the first leaf-sheath. Small brown spots were formed on the blade. Most of the seedlings showing the above symptoms also died.

(ii) Damage to mature plants

The fungus penetrates the leaf tissue through the stoma or directly into the epidermal cells as previously recorded by Nisikado and Miyake (1922), Ocfemia (1924a, 1924b) and Tullis (1935). The mycelium grows intercellularly through the photosynthetic areas and intracellularly through the vascular bundles. Tullis (1935) has recorded that the bundle sheaths of resistant varieties are less readily penetrated by the fungus as they interfere with the lateral spread of the invading mycelium ; secondly, in the resistant varieties the fungus is hemmed in by the formation of deposits which accumulate in the intercellular spaces.

(iii) Damage to seeds

The spots in the grains are frequently very deep as the mycelium penetrates the palae and lemmae. According to Tucker (1922) the brown spots in the grains are so deep that even polishing fails to remove the spots in the grains. Ocfemia (1924a) records that *Helminthosporium oryzae* overwinters as a dormant mycelium in the lemmae and palae, or even on the outer surface of the ovary walls, being attached at these situations by means of star shaped appressoria. Officers of the department of Agriculture in Bengal reported that rice obtained from badly infected crop had a distinctly unpleasant taste.

EXTENT OF DAMAGE

(i) Loss in weight and loss in germination of seeds affected by the disease

Lots of 100 "healthy" and 100 "spotted" seeds of each sample were separately weighed and their weights averaged for comparison. The germination of these "healthy" and "spotted" seeds was tested in petri dishes. The data are presented in Table III.

From the data it can be noted that a distinct loss in weight and germination are associated with the diseased condition of the seeds. A correlation between loss in germination and the proportion of infected grains in a sample could not however be established. The degree of infection as well as other factors affecting germination, like storage, have to be standardised before such a correlation can be established.

TABLE III

Showing the loss in weight and loss in germination sustained by rice grains due to infection by H. oryzae

Variety of paddy	Source of collection	Weight of 100 healthy grains in grams	Weight of 100 diseased grains in grams	Germination of H. grains %age	Germination of d. grains %age	Percentage of disease in sample	Germination of sample %age
Dudsar	Mayanagari ..	2.48	1.92	99	63	46	87
„	Alipur	..	..	..	..	30	48
„	Doors	..	..	..	..	..	..
„	Jalpaiguri ..	2.64	2.26	72	62	29	72
„	Ishurdi	2.80	2.35	98	74	21	91
„	Rajshahi ..	2.70	2.56	96	20	10.7	95
„	Naogaon ..	2.80	2.50	100	96	14.7	100
„	Rangpur ..	2.62	2.41	92	84	17.1	87
„	Gaibanda ..	2.64	2.37	100	80	10.4	84
Indrasail ..	Hajigang.. ..	2.43	1.81	87	72	80	80
„	Comilla	2.65	2.00	97	85	6	90
„	Mayanagari	2.50	1.89	97	81	62	84
Patnai 23 ..	Chinsura ..	3.21	2.79	97	62	5	92
„	Tamluk	2.65	2.37	98.7	80	7	91
„	Amta	2.70	2.20	..	..	12.7	90.7
„	Domjur	2.76	2.00	87	65	45	82.7
Jhingasail ..	Bishnupur ..	2.50	2.32	91.6	81	11.3	86.4
„	Bankura ..	2.30	2.10	98.4	59	9.3	90.3
„	Midnapur ..	2.20	2.15	92.8	92.5	10.3	92.2
„	Jhargram ..	2.20	1.80	82	59.4	14.	77
„ ..	Amta	2.2	2.10	..	..	12.5	93.6
„	Domjur	2.2	1·70	91.9	67	10	90.6
„	Rajshahi ..	2.6	2.30	96	20	10.7	95
Bhashamanik ..	Chinsura ..	1.94	1.78	98	80	..	..
„ ..	Bishnupur ..	1.9	1.80	97.5	43.6	12.5	88.5
„ ..	Bankura ..	1.80	1.50	94.9	68.8	9.6	87.5

TABLE III—*concld.*

Variety of paddy	Source of collection	Weight of 100 healthy grains in grams	Weight of 100 diseased grains in grams	Germination of H. grains %age	Germination of d. grains %age	Percentage of disease in sample	Germination of sample %age
Bhashamanik ..	Midnapur ..	1.90	1.50	86.5	75	7.6	83.9
,, ..	Jhargram ..	1.70	1.50	85.2	64.2	10.5	84
,, ..	Amta	1.80	1.60	91	85.5	9.8	88
Bhashamanik ..	Domjur	2.00	1.70	90.2	77.5	11.7	88.9
Nonaramsail ..	Bishenpur ..	1.50	1.40	89.4	78.4	13.2	87.8
,, ..	Bankura ..	1.40	1.30	83.4	79	12.5	81.7
,, ..	Jhargram ..	1.40	1.30	89.2	73	10.8	74.3
,, ..	Amta	1.30	1.25	..	..	11.2	91.8
,, ..	Domjur	1.30	1.20	88.1	64.4	10	84.6
Sindurmukhi ..	Bankura ..	2.2	2.00	79.1	67.2	23.4	76.2
Dhariyal ..	Mayanagari ..	3.28	2.47	42	30	39	39
,, ..	Midnapur ..	2.50	2.14	91	85.2	27.2	87.5
,, ..	Bankura ..	2.40	2.14	98	95.7	4.5	98
Nagra	Midnapur ..	1.60	1.40	..	..	..	..
,, ..	Chinsura ..	1.90	1.79	100	76	4	95
Latisail ..	Brahmanbaria ..	2.55	2.42	97	86	17	88
,, ..	Mayanagari (Seed Store).	2.58	2.08	99	68	80	63
,, ..	Mayanagari (Farm)	2.63	2.58	92	65	56	75
,, ..	Hajigang ..	2.43	1.81	93	73	9	90
,, ..	Chinsura ..	2.64	2.48	99	78	22	80
,, ..	Comilla	2.70	2.30	97	75	10.2	88

(iii) **Field trials**

An experiment was conducted at the Rice Research Stations at Bankura and at Chinsura, in 1944-45, to determine the loss in germination and the loss resulting from sowing diseased seeds in the yield of paddy and straw. As mentioned above seeds of "Latisail" which had 56% spotted grains were obtained from the Mayanagari Farm. Five treatments were included *viz.*, (1) completely healthy seeds ; (2) seeds with 10% diseased grains mixed ; (3) seeds with 30% diseased

grains mixed ; (4) seeds with 50% diseased grains mixed ; (5) wholly diseased seeds. In order to prepare the grades of diseased seeds they were separated into "spotted" and "healthy" seeds and subsequently mixed in the required proportion by weight.

Loss of germination in field trials

The seeds were sown in the seed-beds on 28-6-44 at Chinsura. Five adjacent beds were sown with the five types of seeds. The size of the seedling beds was 18′ x 8′. The germination was recorded in the beds by counting the number of seedlings in two areas of three feet square, chosen at random in the beds. The data are presented in Table IV. It will be seen that the spotted condition of the seeds did not influence the germination under field conditions.

TABLE IV

Comparative germination records of diseased and healthy seeds in the seedling beds at Chinsura

Treatment	Total No. of seedlings in two square of one sq. yard each	Total No. of seedlings infected by *H. oryzae* in the squares
100% healthy seeds	2336	3
10% diseased seeds + 90% healthy seeds ..	2452	....
30% diseased seeds + 70% healthy seeds ..	2077	....
50% diseased seeds + 50% healthy seeds ..	2228	....
100% diseased seeds	2309	3

At Bankura the seeds had to be germinated in bags and then transferred to the beds.

Loss in the yield of paddy and straw by sowing spotted grains

The seedlings were transplanted to the experimental plots on 17-8-44 at Chinsura and on 4-8-44 at Bankura. The five treatments were replicated six times so that there were six randomised blocks of five plots each. The size of the unit plot at Chinsura was 20′ x 12′ while the plot size at Bankura was 14′ x 10′, with a bund of two feet between the plots. The experiment was harvested in the first week of December at both the places. A border of one foot was left in all the plots to avoid border effects. An analysis of the data for Chinsura is

presented in Tables V and VI and that of Bankura in Table VII and VIII. From the analysis of both the experiments it is seen that there were no significant differences between the treatments. Sowing spotted and diseased seeds was not followed by any significant losses in yield.

TABLE V

Analysis of the yield of paddy by sowing healthy and diseased seeds at Chinsura

Source	Sum of squares	Degrees of freedom	Variance	F
Between blocks	1217.46	5	243.492	7.74
Between treatments	263.46	4	65.865	2.12
Error	629.54	20	31.477	
Total	2111.46	29		

TABLE VI

Analysis of the yield of paddy straw by sowing diseased and healthy seeds at Chinsura

Source	Sum of squares	Degree of freedom	Variance	F
Between blocks	3028	5	605.6	11.04
Between treatments	190.13	4	47.5325	1.06
Error	897.87	20	44.89	
Total	4116.0	29		

TABLE VII

Analysis of the yield of paddy by sowing diseased and healthy seeds at Bankura

Source	Sum of squares	Degrees of freedom	Variance	F
Between blocks	1906.942	5	381.388	1.749
Between treatments	265.667	4	66.417	
Error	4361.433	20	218.072	
Total	6534.042	29		

TABLE VIII

Analysis of yield of straw by sowing diseased and healthy seeds at Bankura

Source	Sum of squares	Degrees of freedom	Variance	F
Between blocks	281.1	5	56.42	
Between treatments	130.5	4	32.62	
Error	4162.0	20	208.1	
Total	4574	29		

Loss in yield in relation to the intensity of infection by H. oryzae

Though the disease did not appear as an epiphytotic, it was observed that most of the plants or stools were infected by *Helminthosporium oryzae*. The relative intensity of infection of the disease in the experimental plots was recorded both at Bankura and at Chinsura. For this purpose two plots, each of nine square feet, were selected at random in a plot and observation made on the number of stools infected, the intensity of spotting (mild, moderately severe, and severe) and the parts of plant affected (leaf, sheath and ears). The data for Bankura and Chinsura are presented in Table IX. The infection was mild in all the plots but there was a difference in the percentage of infection between the plots. The data, however, show that the amount of infection did not influence the yield when infection was mild.

TABLE IX

Percentage of infected stools, and the yield of paddy and straw (in ounces) in thirty experimental plots at Bankura and Chinsura

Plot No.	Bankura			Chinsura		
	Percentage of infected stools	Yield of paddy	Yield of straw	Percentage of infected stools	Yield of paddy	Yield of straw.
1	25	53	58	71	128	136
2	100	88	79	62.5	124	152
3	100	67.5	67	56.5	144	144
4	100	49	54	65.6	126	144
5	100	65	64	74.2	128	136
6	75	67	68	74.2	116	116

TABLE IX—*concld.*

Plot No.	Bankura			Chinsura		
	Percentage of infected stools	Yield of paddy	Yield of straw	Percentage of infected stools	Yield of paddy	Yield of straw
7	100	42	46	84.3	112	116
8	100	61	55	80.0	112	120
9	37.5	70	74	64.5	104	112
10	100	100	91	61.3	120	120
11	50	54	54	90.0	108	112
12	56.3	97	91	50.0	124	128
13	81.3	72	78	67.8	132	120
14	12.5	51	55	87.1	120	120
15	87.5	71	72	78.3	112	116
16	18.8	73	82	50.1	116	120
17	62.5	61.5	60	57.6	104	112
18	18.8	78.5	75	74.4	124	112
19	81.3	85	74	93.8	104	112
20	31.3	63	77	65.6	112	104
21	12.5	48	53	64.5	120	112
22	87.5	46	65	75.0	120	120
23	87.5	48	65	62.5	112	104
24	25.0	57.5	77.5	80.7	120	104
25	93.8	52	62	61.3	136	132
26	100.0	57.0	66	75.9	140	116
27	81.3	61.5	68	68.8	112	124
28	93.8	50	66	67.7	124	120
29	87.5	55	77	77.4	124	120
30	68.8	49	71	59.4	108	120

Loss caused by Helminthosporium oryzae in epiphytotic years

In the 1942-43 season, serious losses were recorded in the districts of Birbhum, Bankura, Midnapur, Hoogly, Howrah and 24 Paraganas. Though actual statistics are not available to estimate accurately the losses sustained, a comparison of the yields on the Govt. Farms shows that very low yields were recorded in that year. In Table X, the yields per acre of all the varieties of paddy cultivated in the Rice Research Stations, Bankura and Chinsurah, in 1942-43 are compared with the corresponding yields of the varieties in the previous year, which was a normal year as far as the yield of paddy was concerned.

CONCLUSION

The nature of damage caused by the disease under Bengal conditions is comparable to the damage caused by it in other countries, like Japan, Philippines, Puerto Rico, U.S.A. etc. Thus seedling blight, leaf and grain-spots have been observed here as well as elsewhere. The fungus invades the leaf either through the stoma or directly into the epidermal cells. The mycelium grows intercellularly through the photosynthetic areas and intracellularly through the vascular bundles. The spots on the grains at the region representing the invaded tissue of the grains go deep through the palae and lemmae.

Though the seedling blight has been observed, seed-borne primary infection is very rarely met with and from field observation and experimental evidence presented above, it may be said that seedling blight phase of the disease is not likely to cause any extensive damage to rice in Bengal. However, when epiphytotic of the disease occurs, almost a total destruction of the crop is to be expected. None except "Kataktara" amongst the twenty-three varieties cultivated in the Rice Research Stations in Bengal was found to be very resistant to the disease in the 1942-43 season. Total immunity is absent in any variety. On the contrary the loss in yield recorded was 50-91% in all the popular and widely cultivated strains.

But in normal years the disease occurs in a mild from. In such a season, wide differences in the amount of infection (as judged from the percentage of infected stools) could not be correlated with any corresponding variation in the yield of straw or paddy.

The diseased condition of the grains is associated with a loss in the weight and germination in petri dishes is lower in diseased than in healthy grains. In the field during sowing time no such differences could be noted.

SUMMARY

A description of *Helminthosporium oryzae* (=*Ophiobolus miyabeanus*) based upon collections from Bengal is given and compared with those of earlier authors.

The nature and extent of damage caused by *Helminthosporium oryzae* in rice in non-epiphytotic years was studied with reference to (i) loss of weight caused by its incidence on the grains, (ii) loss in germination of the diseased seeds as compared with healthy ones under laboratory and field conditions, (iii) loss sustained in the yield of paddy and straw by sowing diseased grains and (iv) the relation between the extent of infection in a crop at the time of harvest and the yield of paddy and straw.

TABLE X

The yield of rice per acre at the Rice Research Stations at Bankura and Chinsurah in the epiphytotic year (1942-43) *compared with the yield per acre in the Stations in a normal year* (1941-42)

No.	Variety of paddy	Bankura							Chinsurah						
		Yield per acre in 1941-42			Yield per acre in 1942-43			Loss in yield (percentage)	Yield per acre in 1941-42			Yield per acre in 1942-43			Loss in yield (percentage)
		mds	srs	chs	mds	srs	chs		mds	srs	chs	mds	srs	chs	
1	Charnok (Aus)	11	35	..	4	34	3	59.15	8	13	11	4	35	1	41.52
2	Dhariyal ,, ...	14	19	..	7	12	2	49.54	14	18	6	7	12	13	49.57
3	Kataktara ,, ...	15	21	3	13	7	..	15.1	13	26	13	13	6	13	..
4	Marichbati ,, ...	14	36	26	7	36	5-5	46.93	14	36	13	7	15	1	50.33
5	Bhutmuri ,, ...	14	3	2	13	24	10	6.83	4	3	1	13	27	3	..
6	DXL (Dular) ,, ...	6	10	12	7	20	..	16.41	..	..	..	..	..	..	..
7	Badkalamkatti (Aman)	16	17	8	9	37	11	39.46	19	..	..	7	20	6	60.47
8	Badshabhog ,, ...	20	26	4	3	18	12	83.2	20	26	6	8	11	10	59.87
9	Chinsura II (Bhashamanik). ,, ...	41	12	4	9	24	12	76.71	27	14	..	7	36	1	79.11
10	Daudkhani ,, ...	18	33	4	1	26	9	91.17	18	34	6	1	26	13	91.14
11	Dudsar ,, ...	23	0	2	6	4	1	73.47	23	..	..	13	37	10	39.47
12	Hingasail ,, ...	27	13	8	3	24	4	86.5	24	25	3	3	14	..	86.39
13	Latisail ,, ...	59	13	12	12	11	2	79.3	31	31	3	12	12	..	61.29
14	Nagra ,, ...	5	6	14	7	14	7	29.73	26	6	13	5	37	3	82.04
15	Patnai ,, ...	30	3	11	3	26	13	87.8	24	26	13	3	26	13	85.12
16	Tilakkatcheri ,, ...	20	16	14	14	20	6	28.87	18	29	10	10	22	1	43.69
17	Indrasail ,, ...	32	16	0	8	10	7	74.5	33	33	10	8	10	7	75.59
18	Bolder ,, ...	26	21	4	3	14	6	87.33	26	21	3	3	14	13	87.29
19	Ajau ,, ...	34	26	2	6	22	8	81.06	34	26	6	6	5	3	82.31
20	Sindurmukhi ,, ...	28	17	4	2	37	0	76.91	25	33	10	2	39	3	88.46
21	Rupsail ,, ...	23	32	10	3	4	5	87.71	23	27	10	3	4	4	86.87
22	Monaramsail ,, ...	18	20	5	4	27	0	74.74	18	20	..	4	25	10	74.81
23	Raghu sail ,, ...	28	0	12	3	20	7	87.4	..	..	..	..	..	..	..

Both the "collar rot" and "damping-off" symptoms were noticed in the seedlings in the field and laboratory. The pathogen penetrates the leaf through the stomata or directly and causes infection. The infection in the grains is sometimes deep seated.

A distinct loss of weight is sustained by both "aman" and "aus" paddy grains when they are infected by *Helminthosporium oryzae*.

Though under laboratory conditions the germination percentage of the diseased seeds was lower than that of healthy ones, no such difference could be observed in field trials. No significant loss in yield was associated with the sowing of diseased seeds. The extent of infection in a field (percentage of stools infected) did not also influence the yield of paddy and straw.

Severe losses in yield were sustained as a result of the epiphytotic outbreak of the disease in Bengal in 1942-43. This is clearly seen when the yield per acre of the 23 varieties of paddy cultivated in the Rice Research Station, Bankura and Chinsurah, in 1942-43 is compared with the yield of the same varieties in the previous season, 1941-42.

Thus the disease causes an appreciable damage to rice only when epiphytotics break out. In other years the fungus behaves like weak pathogen and, though present, does not damage the crop.

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THE CHONDRIOME IN THE GENUS PYTHIUM

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THE term "chondriome" is the name given by Meves to the entire chondriosomal (mitochondrial) contents of a cell or tissue. According to Newcomber (1940) the term "mitochondria" refers to those granules, rods, or filaments in the cytoplasm of nearly all cells which are preserved by bichromates within a pH range approximately between 4·6 to 5·0 and which are destroyed by acids or fat solvents.

The chondriome in fungi was first described in the ascus of *Pustularia vesiculosa* by Guilliermond (1911). Since then it has been described in various groups of fungi ranging from the *Plasmodiophoraceae* to *Autobasidiomycetes*. While the mycelium of some fungi with their relatively broad hyphae afford favourable material for the observation of the chondriome, that of the genus *Pythium* presents much difficulty due to the thinness of its hyphae, the diameter of which in some species studied varied from 1·7 to 4·8μ (*e.g. Pythium complectens*) to the maximum breadth of 3·8 to 9·8 μ (*e. g. Pythium de Baryanum* var. *pelargonii*).

Dangeard (1931), while studying the cytoplasmic inclusions of *Pythium muscae*, described mitochondria as cystosomes, spherical in shape, and Edson (1915) studied them in *Rheosporangium* (*Pythium*) *aphanidermatum*. Saksena (1932) described for the first time with accuracy the chondriome in the vegetative mycelium of *Pythium de Baryanum*. A little later Joyet-Lavergne (1932) studied the oxydo-reduction power of mitochondria of a species of *Saprolegnia* and *Pythium de Baryanum*, both in their vegetative mycelium and sexual organs. Saksena (1936) made a comprehensive study of the chondriome in four species of the genus *Pythium, viz. P. deliense*, *P. indigoferae*, *P. mamillatum* and *P. de Baryanum*.

The present investigation was taken up with a view to study the structure of mitochondria found in other species of the genus *Pythium*.

MATERIAL AND METHODS

The following 25 species of *Pythium* were obtained from the Centraal-Bureau voor Schimmelcultures, Baarn, Holland :—

P. acanthicum Drechsler, *P. afertile* Kanouse et Humphrey, *P. aphanidermatum* (Eds.) Fitz., *P. araiosporon* Sideris, *P. aristosporum* Vanterpool, *P. arrhenomanes* Drechsler var. *canadensis* Vanterpool et Truscott, *P. artotrogus* (Mont.) de Bary, *P. ascophallon* Sideris, *P. complectens* Braun, *P. de Baryanum* Hesse, *P. de Baryanum* Hesse var. *pelargonii* Braun, *P. deliense* Meurs, *P. diameson* Sideris, *P. epiphanosporon* Sideris, *P. graminicolum* Subramaniam, *P. hyphalosticton* Sideris, *Pythium indigoferae* Butler, *P. leiohyphon* Sideris, *P. leucosticton* Sideris, *P. mamillatum* Meurs. *P. polyandron* Sideris, *P. rhizophthoron* Sideris, *P. scleroteichum* Drechsler and *P. spaniogamon* Sideris.

The mycelium for the supra-vital study was obtained by growing the fungi in 50 cc. Erlenmeyer flasks containing about 30 cc. peptone solution (1%) kept at

25° C. The mycelium was taken from young colonies and irrigated under the microscope with various dyes dissolved in Ringer's solution consisting of :—

NaCl	6.0 gm
KCl	0.075 gm
CaCl	0.1 gm
$NaHCO_3$..	0.1 gm
Distilled water	1000 cc

The vegetative mycelium was studied both in the living and fixed materials. The fixatives and the technique used were the same as employed by the senior author, Saksena (1936).

OBSERVATIONS IN THE LIVING MATERIAL

When the mycelium, growing in 1% solution of bacto-peptone, was examined under the high power of the microscope, it was not possible to see mitrochondria in the hyphae due to their thinness ; only fat particles were seen moving rapidly. Without staining mitochondria were seen, however, in the cytoplasm with careful observation under the oil immersion lens. They were usually in the form of granules, short rods or filaments (fig. 1).

The filamentous forms were slender bodies of varying length lying mostly parallel to the longitudinal axis of the filament. The mitochondria of various forms were of nearly the same refraction as that of the cytoplasm and moved very sluggishly in it. The granular forms could easily be distinguished from the fat particles because they moved sluggishly in the cytoplasm and were less refrigant than the fat particles.

The following were the different forms of mitochondria found in the various species studied :—

I MOSTLY GRANULAR—in *P. acanthicum*, *P. afertile*, *P. aphanidermatum*, *P. aristosporum*, *P. de Baryanum* and *P. mamillatum*.

II MOSTLY ROD SHAPED—in *P. arrhenomanes*, *P. diameson* and *P. leiohyphon*.

III MOSTLY FILAMENTOUS—in *P. complectens*, *P. epiphanosporon*, *P. graminicolum*, *P. indigoferae*, *P. polyandron* and *P. scleroteichum*.

IV GRANULAR AND ROD-SHAPED MIXED—in *P. artotrogus*, *P. de Baryanum* var. *pelargonii* and *P. spaniogamon*.

V GRANULAR, ROD-SHAPED AND FILAMENTOUS MIXED—in *P. araiosporon*, *P. arrhenomanes* var. *canadensis*, *P. ascophallon*, *P. deliense*, *P. hyphalosticton*, *P. leucosticton* and *P. rhizophthoron*.

The authors could not find any species where there were exclusively granular mitochondria. The granular form was present in all species especially at the tips, where in some places they were seen elongating to assume rod-shaped or filamentous forms. They become thinner and thinner and elongate. This seems to indicate that they are formed by the elongation of the granular mitochondria. In a few species *e.g. P. aphanidermatum* (fig. 2) the granular form was predominent, while the other two forms were rare. In others *e.g. P. leiohyphon* (fig. 3) the rod-shaped mitochondria were in greater number than the other forms ; while in some *e. g. P. epiphanosporon* (fig. 1) the number of filamentous ones was much larger. In

P. rhizophthoron (fig. 4) all the three forms of mitochondria were nearly equal in number. In *P. spaniogamon* (fig. 5) only rod-shaped and granular forms were nearly equal in number, the filamentous form being absent.

More often filamentous mitochondria moved continually under the influence of cytoplasmic currents, and at times they became stationary. Some times they were seen forming ramifications which were transitory. Such ramifications are formed, as explained by Guillermond (1941, p. 65), when mitochondria encounter an obstacle such as fat globules or other structures. At times they were seen fragmenting. During this process they became stretched and dumbel-shaped getting thinner and thinner in the middle where they finally fragmented into two (fig. 4.).

SUPRA–VITAL STAINING

Mitochondria become more clear when stained with Janus green, Höcht B, and dahlia violet in very minute doses (0.001%). To stain mitochondria it took about 10-15 minutes. With Janus green they took up a sky blue colour, while dahlia violet imparted a light violet tinge. Their structure after being stained was nearly the same as described when no stain was employed, but many of them appeared in the process of fragmentation and vesiculisation due to the effect of dyes when used in concentrations higher than 0.005%. During the latter process the rod-shaped and filamentous mitochondria became shorter in length. The various forms then swelled up and finally transformed themselves into vesicles (fig. 6). In the centre of each a clear space was seen. The Janus green which at first was taken up as a sky blue stain, later on turned pink. This was due to the formation of a reduction product of the dye. This phenomenon has alrady been reported by Guilliermond and Gautheret (1938, 1939) Saksena (1932,) Murdia (1938) and by Saksena and Bhargava (1941), in various fungi.

OBSERVATION ON FIXED MATERIAL

Of all the fixatives used, Helly's liquid was found to be the most satisfactory, though sublimé-formol solution also gave very good results in some cases.

Mitochondria were stained black while the cytoplasm remained nearly colourless. The structure of mitochondria was found to be the same as studied in the living condition.

The size of granular mitochondria varied from 0.25 μ in diameter in *P. aristosporum*, to 2 μ in *P. mamillatum*, that of the rod-shaped from 0.5 μ in length in *P. spaniogamon* to 2 μ in *P. rhizophthoron*, and filamentous from 1 μ in length in *P. leucosticton* to 4 μ in *P. epiphanosporon*.

INFLUENCE OF TEMPERATURE

The authors exposed the mycelia of *P. araiosporon* and *P. rhizophthoron* to different temperatures at 40°, 45°, and 55° C and fixed them in Helly's fixative. The preparations were then stained with haematoxylin and mounted *en masse*. It was found that even the temperature of 55° C did not destroy mitochondria (fig. 8). No doubt, they became thinner and were seen oriented in irregular manner instead of being parallel to the longitudinal axis of the hyphae. At some places many were vesiculised (fig. 8).

For a long time mitochondria were considered to be sensitive to high temperatures. Policard and Mangenot (1922) reported that the temperature of 45° to 50° C

destroyed the chondriome instantaneously in animals and in some species of the family *Saprolegniaceae*. But later on, it was found that in the *Saprolegniaceae* (Guilliermond, 1941, p. 66) mitochondria merely became less distinct at 45°—50° C on account of the modification of the viscosity of the cytoplasm, and at the same time they became altered, *i.e.* fragmentation into balls and then transformation into vesicles took place. Their chromacity decreased but they persisted up to 58° C. Saksena (1936) got similar results in *Pythium deliense*.

EFFECT OF SOME REAGENTS

Bits of mycelium of *P. araiosporon*, and *P. rhizophthoron* were placed in glacial acetic acid, saturated solution of picric acid, absolute alcohol and commercial formalin for 1 hour and were then fixed with Helly's liquid. The effect of these reagents was not very appreciable. In fact the mitochondria in hyphae treated with picric acid and formalin (fig. 9) were well preserved. On account of alcohol, the cytoplasm got shrunken within the hyphae. Due to this change, observation became difficult and at places impossible. Wherever plasmolysis was least, mitochondria were seen in various aspects, *i.e.* granular, rod-shaped, filamentous or vesiculised. Alcohol and acetic acid did not seem to produce much change (figs. 10 and 11). In no case the hyphae were seen devoid of mitochondria.

Mitochondria are said to consist of lipoproteid complex in which lipoids predominate and it is generally reported that in plants and animals they, on account of their chemical composition, get dissolved in fixatives containing alcohol and acids. Guilliermond has reported that in the *Saprolegniaceae* they get profoundly altered if treated with fixatives containing alcohol and acids but do not get entirely dissolved.

Milovidov (1929), in the case of *Saprolegnia* sp. found that formalin fixed, acetic acid altered and absolute alcohol dissolved the mitochondria. Saksena (1936) treated the mycelium of some species of *Pythium* with the liquids of Bouin (containing acetic acid) and Lenhossek (containing both absolute alcohol and acetic acid) and found that these reagents did not profoundly affect mitochondria. It seems that their constitution is somewhat different from that of normal ones.

EFFECT OF KOLATCHEV'S FLUID

The Kolatchev's method modified by Bowen (1929-30) recommended to bring about the Golgi apparatus was tried with the mycelium of *P. araiosporon* and *P. rhizophthoron*. It was kept in 2% osmic acid for 7 and 15 days at 40° C. It was found that mitochondria got blackened. Vesiculisation of these elements was very profound in the hyphae kept for 15 days (fig. 12). Saksena (1936) has reported similar results in other species of *Pythium*. The material was found unsuitable for the study of the so called 'Golgi bodies', since the hyphae were thin and vacuoles small.

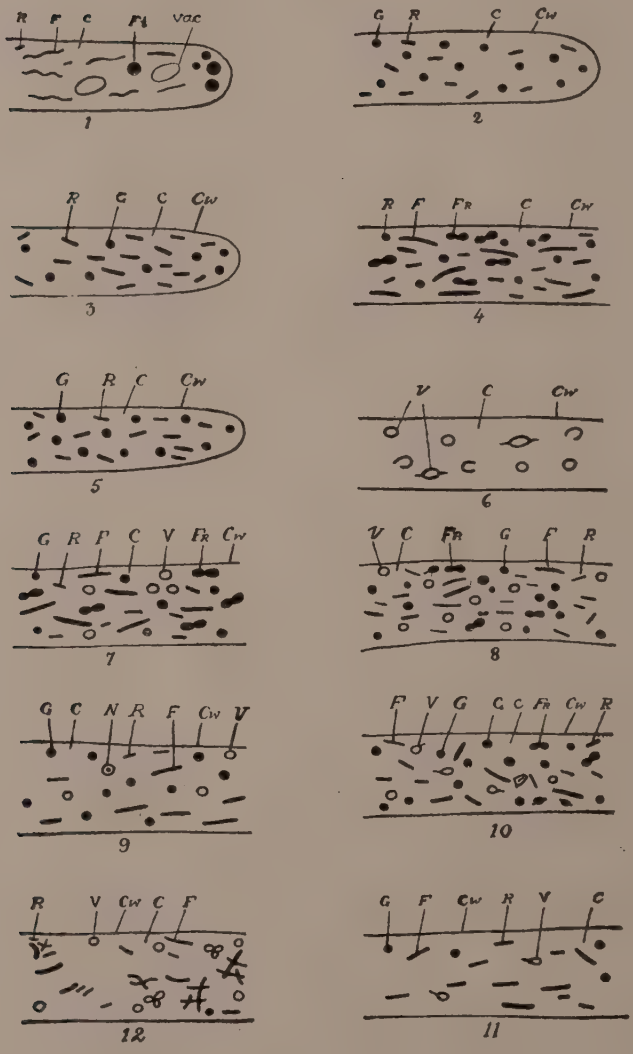
SUMMARY

1. The chondriome of 25 species of *Pythium* was investigated. All the three forms of mitochondria, *i.e. granular, rod-shaped* and *filamentous* were present in varying proportions.

2. Picric acid and formalin had no marked effect on mitochondria.

3. The temperature of 55° C. did not destroy mitochondria.

4. With Kolatchev's technique mitochondria were preserved. Vesiculisation was more marked in hyphae kept in the fixative for 15 days.



Saksena & Bose del.

Plate I. See legend at the end of the article

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EXPLANATION OF PLATE

Abbreviations used.—C—cytoplasm ; Cw—cell wall ; F—filamentous mitochondria ; Fr—fragmenting mitochondria ; Ft—fat particles ; G—granular mitochondria ; N—nucleus ; R—rod shaped mitochondria ; V—vesiculised mitochondria ; Vac—vacuole.

Figures unless otherwise stated are drawn from preparations fixed in Helly's fluid and stained with iron alum haematoxylin. (X 3000).

Fig. 1.—A portion of the hypha of *P. epiphanosporon*, showing fat globules, vacuoles; filamentous, rod-shaped, granular and serpentine mitochondria (in living condition)

Fig. 2.—A portion of hypha of *P. aphanidermatum*, showing granular mitochondria (in living condition)

Fig. 3.—A portion of the hypha of *P. leiohyphon*, showing mitochondria mostly rod-shaped

Fig. 4.—A portion of hypha of *P. rhizophthoron*, showing granular, rod-shaped, and filamentous mitochondria mixed

Fig. 5.—A portion of hypha of *P. spaniogamon*, showing granular and rod-shaped mitochondria mixed

Fig. 6.—A portion of hypha of *P. epiphanosporon*, stained supra-vitally with Janus green showing mitochondria vesiculising (in living condition)

Fig. 7.—A portion of hypha of *P. araiosporon*, showing granular, rod-shaped and filamentous mitochondria mixed

Fig. 8.—A portion of hypha of *P. rhizophthoron* kept at 55° C. for 18 hours and then fixed in Helly's fluid, showing granular, rod-shaped and filamentous mitochondria mixed. The mitochondria are oriented in various directions

Fig. 9.—A portion of the hypha of *P. rhizophthoron*, treated with saturated solution of picric acid for 1 hour and then fixed in Helly's fluid, showing granular, rod-shaped and filamentous mitochondria mixed

Fig. 10.—A portion of hypha of *P. rhizophthoron*, treated with glacial acetic acid for 1 hour then fixed in Helly's fluid, showing granular, rod-shaped and filamentous mitochondria mixed

Fig. 11.—A portion of hypha of *P. rhizophthoron*, treated, with absolute alcohol for 1 hour and then fixed in Helly's fluid, showing granular, rod-shaped and filamentous mitochondria mixed

Fig. 12.—A portion of hypha of *P. rhizophthoron*, after 15 days osmification with Kolatchev's technique, showing mitochondria anastomosing, and numerous vesiculised mitochondria

STUDIES ON INDIAN ASPERGILLI

By U. N. MOHANTY

(Accepted for publication May 15, 1948)

On account of their common occurrence in almost all kinds of substrates, the members of the genus *Aspergillus* have received attention from the taxonomists from comparatively early times. In India, studies from the taxonomic view-point were first made by de Mello (1920) and then by de Mello and Carmo Vas (1921) of Nova Goa in the Portuguese Indian colony of Goa. The next publication is that by Chaudhury and Umar in 1938. Besides these, the *Aspergilli* are referred to here and there in Indian literature in the course of general studies on the fungus flora of soil, air, dung and other substrates. Thus the bulk of literature on Indian *Aspergilli* is indirect in nature and our knowledge of them must be gathered from scattered references. In this way, references have been found to 33 species and two varieties of *Aspergilli*; but in view of the fact that the species of *Aspergillus* have now been studied in greater detail in foreign countries, particularly by Thom and Church (1926) and by Thom and Raper (1939, 1941, 1945) and as they have gained importance because many of the species have yielded valuable antibiotics, a systematic study of this genus in India seemed desirable.

SPECIES OF ASPERGILLUS RECORDED FROM INDIA AND THEIR STATUS

A list of *Aspergilli* recorded for India is given by Butler and Bisby (1931) and by Mundkur (1938). They have not mentioned some species which had already been reported; others have been recorded subsequent to the publication of their monographs. The following list of species of *Aspergillus*, with the authors first recording them from India, is believed to be complete.

TABLE I *Species of* Aspergillus *recorded for India*

No.	Name of the species	Name of recording author
1	?aguiari de Mello & Vas	de Mello & Vas (1921)
2	?albicans de Mello & Vas	de Mello & Vas (1921)
3	*calyptratus* Oudemans	Chaudhury & Sachar (1934)
4	**candidus** Link ex Fr.	de Mello & Vas (1921)
5	*castanea* Patterson	Butler (1914)
6	**chevalieri** (Mang.) Thom & Church	Galloway (1936)
7	*corolligena* Massee	Massee (1910)
8	*ferruginea* Cooke	Cooke (1880)
9	**flavipes** (Bain. & Sart.) Tom & Church	Chaudhury & Sachar (1934)
10	**flavus** Link ex Fr.	Hutchinson & Ayyar (1915)
11	**fumigatus** Fresenius	Butler (1916)
12	*fumigatus* var. *tumescens* Blum.	Chaudhury & Umar (1938)
13	*fuscus* Amons	Thakur & Norris (1928)
14	?herbariorum (Wigg.)	de Mello (1920)
15	?herbariorum var. intermedia de Mello and Vas.	de Mello (1921)

TABLE I *Species of* Aspergillus *recorded for India*—concld.

No.	Name of the species	Name of recording author
16	*humicola* Chaudhury & Sachar	Chaudhury & Sachar (1934)
17	**japonicus** Saito	Prasad (1938)
18	**luchuensis** Inui	Chaudhury & Umar (1938)
19	**nidulans** (Ediam) Wint.	Thakur & Norris (1928)
20	**niger** van Tieghem	Hutchinson & Ayyar (1915)
21	**ochraceous** Wilhelm	Galloway (1936)
22	?ortae de Mello & Vas	de Mello & Vas (1921)
23	**oryzae** (Ahlb.) Cohn	de Mello (1920)
24	*phaeocephalus* Durieu & Mont.	Cooke (1878)
24a	**phoenicis** (Corda) Thom	
25	*polychromus* de Mello	de Mello (1920)
26	**repens** (Cda) de Bary & Woron.	Thakur & Norris (1928)
27	*sachari* Chaudhury	Chaudhury and Sachar (1934)
28	**sulphureus** (Fres.) Thom & Church	de Mello (1920)
29	**sydowi** (Bain. & Sart.) Thom & Church	Chaudhury & Sachar (1934)
30	**tamarii** Kita	Mason (1928)
31	**terreus** Thom	Mason (1928)
32	*ustilago* Beck	Wawra (1888)
33	**ustus** (Bain.) Thom & Church	Galloway (1936)
34	**variecolor** (Berk. & Br.) Thom & Raper	Thom & Raper (1939)
35	**versicolor** (Vuill.) Tiraboschi	Chaudhury & Sachar (1934)
	NEW RECORDS MADE BY THE AUTHOR	
36	**amstelodami** (Mang.) Thom & Church	
37	**fischeri** Wehmer	
38	**rugulosus** Thom & Raper	
39	**unguis** (Emile-Weil & Gaudin) Thom& Raper	

(Species in **bold** type are valid records and those in *italics* are either synonyms or species considered to be not valid. Doubtful records are in lower case type and preceded by an interrogation mark.)

Of the 33 species and two varieties recorded for India, a large number are either synonyms or invalid species. The material which formed the basis for establishment of *Aspergillus albicans*, *Aspergillus ortae* and *Aspergillus aguiari* as new species by de Mello and Vas (1921), is not now available and the descriptions are not adequate and precise. These species have not been accepted by Thom and Raper (1945) either and we may have to wait till they are re-isolated before they can be accepted as valid species. They are therefore doubtful records.

Aspergillus calyptratus recorded by Chaudhury and Sachar is not accepted as a valid species by Thom and Raper (1945) and Neill (1939), who regard it as synonymous with *Aspergillus fumigatus*. *Aspergillus castanea* recorded by Butler

(1914) for India is not also accepted and relegated by Thom and Raper (1945) to *Aspergillus tamarii* series. *Aspergillus corolligena* is rejected by the same authors as it is not recognizable from the description which alone exists. Both Thom and Church (1926) and Thom and Raper (1945) state that *Aspergillus ferruginea* is not an *Aspergillus* at all. The existence of *Aspergillus fumigatus* var. *tumescens* is doubted by Thom and Church (1926) and it is therefore merged into *Aspergillus fumigatus* by Thom and Raper (1945).

Precise identification of *Aspergillus herbariorum* and its variety *intermedia* is not posssible. The former is considered to be identical with *Aspergillus glaucus* by Neill (1939) and with the *Aspergillus glaucus* group by Thom and Raper (1945). *Aspergillus glaucus* is a composite species and divisible into many species on the size and characters of the ascospores. As de Mello and Vas (1921) who reported the former for India, and established the variety *intermedia*, do not mention the characters of the ascospores, it is not possible to identify their species with precision and both of them are left as doubtful records.

Blochwitz (1939) considers Chaudhury and Sachar's (1934) *Aspergillus humicola* to be same as *Aspergillus flavus* whereas Neill (1939) thinks that it is synonymous with *Aspergillus versicolor*. In the absence of a culture of Chaudhury and Sachar's fungus, its status as a species is considered doubtful and it is also relegated to synonymy. Thom and Church (1926) do not consider *Aspergillus polychromus* as a valid species and, as the data given are not sufficient, they are unable to say whether de Mello had *Aspergillus nidulans* or *Aspergillus sydowi*. This species is therefore merged into synonymy. *Aspergillus ustilago*, according to the same authors, is a synonym of *Aspergillus phœnicis*. *Aspergillus phœocephalus* recorded for India by Cooke (1878) has also been merged into *Aspergillus phœnicis* by them.

If the above synonyms or invalid species are eliminated, the number of species of *Aspergillus* recorded for India stands at 20 to which may be added four more found by the present author, in the course of these investigations. The existence of five species, *viz.*, *Aspergillus albicans*, *Aspergillus aquiari*, *Aspergillus herbariorum* and its variety *intermedia* and *Aspergillus ortae* has been left in doubt. The position of these species and of *Aspergillus sachari* and *Aspergillus humicola* could have been validated if cultures of these species were available.

MATERIAL AND METHODS

Studies were undertaken with 52 cultures, which on critical examination were found to represent only 19 species. Out of these, four species are new records for India and two, namely *A. variecolor*, and *A. nidulans* were known in a different sense. For these two species, which are existing records, it was felt necessary to give correct labels. Therefore in the following account, the descriptions of only six species are given. The representative cultures of the remaining 13 species, which were encountered in course of these studies and the descriptions of which are not given here in order to avoid repetition, have been preserved in the culture collections of the Division of Mycology, Indian Agricultural Research Institute at New Delhi.

Throughout the present study Thom and Church's (1926) technique in identifying the cultures was closely followed and as recommended by them Czapeck' synthetic agar was used as a medium.

DESCRIPTIONS OF THE SPECIES

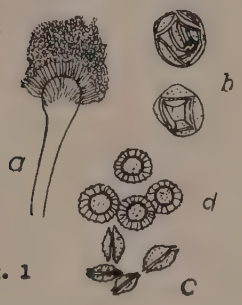

Fig. 1

(1) **A. fischeri** Wehmer (Fig. 1)

Thom, & Church, ... *Amer. J. Bot.* **5** : 84-104, 1918.

Thom, & Church, ... "The Aspergilli' " 1926.

Neill, ... *Trans. and Proc. Roy. Soc. N. Z.* **69**:237-264, 1939.

Colonies in Czapeck's solution agar lanose, becoming velvety with age, spreading, glaucous green to light porcelain green at first, turning to dark porcelain green with age. Reverse at first colourless, turning to light salmon-orange with age. Stalks 190-300×4-8μ ; averaging 248×6.4μ ; continuous, sinuous, bluish in colour specially in the upper part along with the vesicle and sterigmata ; arising from submerged hyphae with foot cells indistinct. Vesicles globose, 17-28μ, averaging 21.8μ in diameter. Sterigmata in one series, closely packed all over the vesicle and directing upwards parallel to the axis of the stalk, 6-8×1-3μ; averaging 7.1×2.5μ. Conidia bluish green in mass, globose to sub-globose, often slightly elliptical, smooth, 2.5-3×1.5-2μ averaging 2.3×1.6μ; conidial chains in closely packed columns ; columnar masses 87-350×35-52μ, averaging 210×42μ.

Perithecia formed abundantly at the temperature of about 45°F, white 175-437μ, averaging 315μ; asci 8-spored, sub-globose, 8-12μ, averaging 9.4μ in diameter. Ascospores colourless or slightly yellowish, biconvex with two equatorial crests that are plate-like with margin broadly irregular. Crests up to 1 in width. Ascospores rough, two-frilled at both ends, 5-7×3.0μ averaging 5×3μ.

Culture G. C. 171, in culture collections, Mycological Section, I. A. R. I., New Delhi, India, from surface of plum roots, Haripur Farm, N. W. F. P., by P. R. Mehta on 27.9.38.

This species is recorded for the first time in India.

Thom and Church (1918) considered *A. fischeri* as a member of the *A. fumigatus* group. Neill (1939) also does not attach much importance to the presence or absence of perithecia and considers *A. fisheri* as a synonym of *A. fumigatus* ; but

following Thom and Church (1926) and Thom and Raper (1945) it is considered best to maintain this as a separate species.

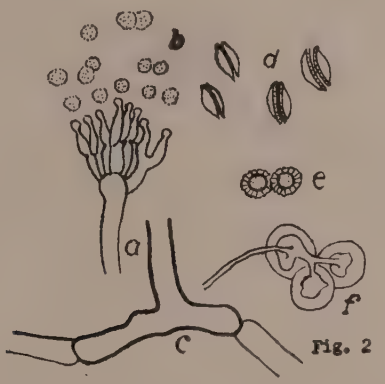

Fig. 2

(2) **A. nidulans** (Eidam) Wint. emend Thom & Raper (Fig. 2)

Thom, and Church, ...	*Amer. J. Bot.* **5**: 84-104, 1918
Thom, and Church, ...	"The Aspergilli" 1926
Thom, and Raper, ...	*Mycologia* **31**: 653-669, 1939
Thakur, and Norris, ...	*J. Indian Inst. Sci.* **11A**: 141-160, 1928.
Chaudhury, and Sachar, ...	*Ann. Mycol.* **32**: 90-100, 1934
Galloway, ...	*Indian J. Agri. Sci.* **6**: 578-585, 1936
Chaudhury and Umar	*Proc. Indian Acad. Sci.* **8**: 79-92, 1939

Colonies in Czapek's solution agar spreading, velvety, dirty-green, passing to light cress-green to dark cress-green in old cultures. Reverse purple red turning to brownish with age. Stalks sinuous, smooth-walled, cinnamon-brown, 51-128 × 2-5μ, averaging 70×3μ, vesicles globose, 7-10μ, averaging 8.0μ in diameter. Sterigmata in two series; primary 5-6×2-3μ, averaging 5-6×2.0μ; secondary 5-6 ×2μ, averaging 6×2μ; conidial chains in columnar masses; columns 52-87 ×26-35μ, averaging 64×32μ; conidia green, globose, rugulose, 3μ.

Perithecia globose, dark-pink when mature, 175-397μ, averaging 306μ, including the hyphal mass surrounding the fruit body; hulle cells abundant; asci 8-spored, ascospores purple-red, lenticular, smooth, with two equatorial crests, 4-5×3μ, averaging 5×3μ, equatorial crests plate-like with smooth margins, nearly 0.8μ in width.

Culture G. C. 304 in culture collections, Mycological Division, I. A. R. I., New Delhi, India, from contaminated petri dish, Mycological laboratory, I. A. R. I., New Delhi, by U. N. Mohanty, on 8-11-41.

A. nidulans has long been known in India ; but since the revision of the *Nidulans* group by Thom and Raper (1939), *A. nidulans* in its restricted sense has not been reported hitherto from this country.

Fig. 3

(3) **A. rugulosus** Thom and Raper (Fig. 3).

Thom, and Raper, ... *Mycologia* **31**: 653:669, 1939

Galloway, ... *Indian J. Agric. Sci.* **6**:578-585, 1936

Colonies in Czapek's solution agar very slow growing, wrinkled in surface, at first light-green due to the formation of conidial heads, later becoming purple brown with age due entirely to the formation of perithecia. Reverse purple-red. Stalks short, sinuous, olivaceous-brown, smooth-walled, 33-84×3-4μ; averaging 72×4μ; vesicles globose 8×10μ, averaging 9.5μ in diameter. Sterigmata in two series ; primary 6-8×3-4μ, averaging 7×3μ, secondary 6×2-3μ, averaging 6×3μ conidia green, globose, verrucose, 3-4μ, averaging 3.4μ in diameter. Conidial chains in columns.

Perithecia formed very freely at various depths of the medium causing splitting of the medium at the middle, globose, thin-walled, purple, surrounded by a mass of hulle cells. Ascospores purple-red with surface rugulose. 5×3μ, averaging 5×3μ, elliptical in one position and globose in another. Equatorial plates two, with entire sinuate margins, colourless, not exceeding 0.8μ in width.

Culture G. C. 305 in culture collections, Division of Mycology, I. A. R. I., New Delhi, India, from exposed petri dish in Mycological laboratory, by U. N. Mohanty, on 10-10-41.

Galloway (1936) in the description of *A. niaulans*, isolated from the soil, reports the presence of both smooth and rough-walled ascospores. His culture having rough-walled ascospores was probably *A. rugulosus* which was not then recognised as a distinct species. Since the publication of the paper by Thom and Raper (1939) *A. rugulosus* is regarded as a separate species distinct from *A.*

nidulans. Therefore the species *A. rugulosus* is now recorded for the first time in India.

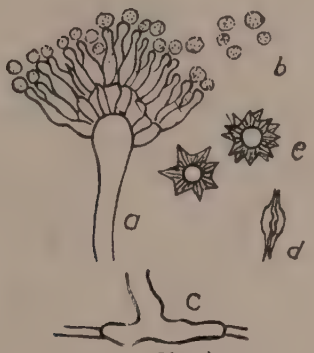

Fig. 4

(4) **A. variecolor** (Berk. and Br.) Thom & Raper, (Fig. 4)

Thom, and Raper, ... *Mycologia*, **31**:653-669, 1939

Saccardo, ... *Syll. Fung.* **7**:154, 1888

Chaudhury, and Mathur,.. .. .. *Ann. Mycol.* **36**: 61-63, 1938

Colonies in Czapek's solution agar spinach green, velvety, slow-growing. Reverse at first colourless, turning to nigrosin violet with the formation of perithecia and finally to dark nigrosin violet with age. Stalks brownish, sinuous, smooth-walled 128-342×2-5μ averaging 260×4μ; vesicle globose to flask-shaped, 8-13μ averaging 10-0 in diameter. Sterigmata in two series ; primary 5×8×2-4μ, averaging 6×3μ; secondary 7×10×2.3μ, averaging 8×2μ; conidia globose, green, smooth when young, becoming rugulose with age, 2-3μ, averaging 2.6 μ in diameter. Conidial chains in columns.

Perithecia globose, either formed in groups or distributed over the colony, supported by a false stalk consisting of hypha and hulle cells ; 437-612μ, averaging 518μ in diameter. Hulle cells generally globose, rarely elliptical slightly yellowish, 13-26μ, averaging 20.8μ in diamter; walls 4-8.5μ, averaging 7.7μ in thickness. Ascus 8-spored, globose, colourless at first, becoming slightly yellow and finally pinkish when the ascospores are ripe, 8×13, averaging 11.4μ in diameter. Ascospores purple-red, lenticular in one position to globose in another, with two equatorial crests up to 3μ in width, which are idented in the margin thus giving a stellate outline to the ascospores, 2.5-4μ, averaging 3.4μ in diamter (excluding the frills).

Culture G. C. 306 in culture collections, Division of Mycology, I. A. R. I., New Delhi, India ; from exposed petri dish cultures, Mycological laboratory, New Delhi, by U. N. Mohanty on 8-10-41.

Chaudhury and Mathur (1938) reported *Emericella medias* as a new species from Lucknow. They did not describe the conidial stage very clearly. Regarding the perithecial stage they describe the perithecial wall to be surrounded by a mass

of air-cells, which are obviously the hulle cells. The ascospores are reported as star-like ; but the measurements are slightly higher than those of *A. variecolor* Saccardo (1888) mentions that Berkeley described *Emericella variecolor* Berk. et Broome from material collected at Secunderabad, South India. Butler and Bisby (1931) however do not mention this species because the taxonomic position of the genus *Emericella* was in doubt. Thom and Raper (1939) have transferred the species to *Aspergillus* as *A. variecolor* and have made all species of the genera *Emericella* and *Inzengaea*, synonymous with *A. variecolor*.

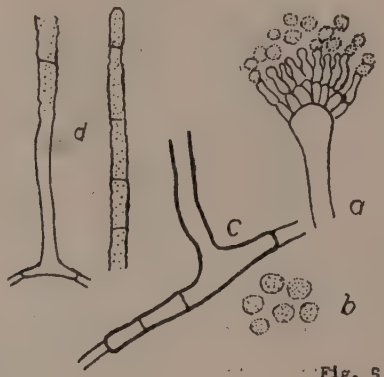

FIG. 5

(5) **A. unguis** (Emile-Weil and Gaudin) emend Thom and Raper (Fig. 5).

Thom, & Raper, ... *Mycologia*, **31**: 653-669, 1939

Colonies in Czapek's solution agar very slow-growing with submerged mycelia plentiful and growing very deep. Conidial formation rather scanty at the centre, becoming profuse towards the margin, which is irregular. Thus the centre of the colony appears white to the naked eye slowly developing to yellowish-green towards the margin, where plenty of conidiophores and conidia are massed. Direct microscopic examination of the petri dish cultures showed the erect conidiophores arising from the submerged mycelium and some sterile, thick-walled hyphae rising among and above the crowded conidiophores. Slide mountings show these sterile aerial hyphæ as brownish, thick-walled, septate, very irregularly roughened at some distances and ending in a blunt point. Sometimes these spicule hyphæ arise from foot cells.

Stalks brown, sinuous, smooth-walled, 40-70×3-5μ, averaging 55×4μ; foot cells sometimes not distinct. Vesicles globose to sub-globose, 7×10μ, averaging 8.3μ; in diameter. Sterigmata in two series; primary 3-6×2-3μ; averaging 5×3μ; secondary 5-7×2-3μ, averaging 6×2μ conidial chains in columns; columnar masses 140-245×35-52μ, averaging 173×40μ; conidia green globose, verrucose 2.5-3μ, averaging 3μ in diameter. The culture is non-ascosporic. No perithecia, or hulle cells observed.

Culture G. C. 307 in culture collections, Division of Mycology, I. A. R. I., New Delhi, India, from exposed petri dish cultures Mycological laboratory, I. A. R. I., New Delhi; by U. N. Mohanty, on 15-9-41.

This species is recorded for the first time in India.

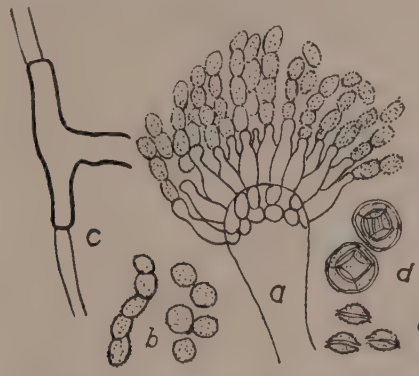

Fig. 6

(6) **A, amstelodami** (Mangin) Thom and Church emend Thom and Raper (Fig. 6).

Thom, & Church, ... "The Aspergilli," 1926.

Thom, & Raper, ... *U. S. Dept. Agric. Misc. Pub.* **426**. 1941.

Colonies in Czapek's solution agar velvety to lanose, very slow-growing, at first pinard yellow to baryta yellow finally turning to deep olive to dark olive with flecks of yellow hyphæ in the central area. Aerial mycelia at first yellowish, turning to brown with age. Reverse and agar brown to black. Conidial heads very few, formed chiefly in the drier areas of the agar slant, glaucus green. Stalks septate, green smooth-walled, 107-385×6-8.5μ, averaging 265×8μ; vesicle globose, 17-26μ, averaging 21.4μ in diameter. Sterigmata in one series, 5-8×2-3μ, averaging ×3μ, conidia green, verrucose, oval, elliptical or globose, mainly elliptical, 5-7μ, averaging 5u in the long axis. Conidial heads radiate splitting into columns in older heads.

Perithecia formed very freely, the culture becoming mainly perithecial and this imparting the colour to the colony. Parithecia yellow, globose, suspended in net-works of hyphea which are yellow at first, changing to brown, sometimes to pink with age. 73-128μ, averaging 102μ in diameter. Asci oblong to globose, at first colourless, changing to yellowish as the ascospores ripen, 8-12μ, averaging 9.6μ in diameter. Ascospores yelowish, lenticular, rough, with deep equatorial furrow and the ridges, 5μ, in the long axis.

Culture G. C. 316, in culture collections, Division of Mycology, I. A. R. I., New Delhi, India ; isolated as a common laboratory contaminant, Mycological laboratory, I. A. R. I., New Delhi, by U. N. Mohanty on 8-10-41.

This species is recorded for the first time in India.

Although Thom and Church divide the *glaucus* group on the characters of ascospores, they do not put much importance on the smoothness or roughness of the ascospores : Moreover they describe the ascospores of *A. amstelodami* as smooth-walled. Thom and Raper (1941) have revised the *glaucus* group and describe that the ascospores of *A. amstelodami* are rough. In the identification of this species Thom and Raper have been followed.

SUMMARY

A list of species and varieties of *Aspergillus* so far recorded from India is given and the status of these species is discussed on the basis of our present knowledge. It is observed that out of 33 species and 2 varieties recorded from India, only 20 species appear reliable.

A detailed account of 6 species collected from India and identified by the author is given. Four of these species, namely *A. fischeri*, *A. rugulosus*, *A. unguis* and *A. amstelodami* are recorded for the first time in India. Thus the total number of Indian Aspergilli at present recognisable is 24 species.

ACKNOWLEDGMENT

My thanks are due to Dr. G. Watts Padwick, formerly Imperial Mycologist, for placing at my disposal, some cultures of *Aspergilli* for examination and for his kind interest in the work. The author is also thankful to Major H. A. Dade, Assistant Director, Commonwealth Mycological Institute, Kew, England, for critically examining the manuscript and supplying the photograph of the original illustration of *A. ustilago* G. Beck, and to Dr. B. B. Mundkur, Chief Mycologist, Bureau of Plant Protection and Quarantines, for reading the manuscript and giving helpful suggestions.

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Fig. 1. *A. fischeri*

(*a*) Mature head. ×400
(*b*) Ascus. × 1000
(*c*) Ascospores with equatorial crests. ×1000
(*d*) Ascospores with crests flat. × 1000
(*e*) Hulle cells. ×400

Fig. 2. *A. nidulans* (Eddam) Wint. emend Thom & Raper.

(*a*) Mature head. ×1000
(*b*) Conidia. × 1000
(*c*) Foot-cell. × 1000
(*d*) Ascospores with two plate-like crests. ×1000.
(*e*) Ascospores with crests flat. ×1000
(*f*) Hulle cells. × 400

Fig. 3. *A. rugulosus* Thom & Raper.

(*a*) Young conidiophore. ×400
(*b*) Mature head. ×1000
(*c*) Conidia. × 1000
(*d*) Ascospores with two equatorial crests. ×1000
(*e*) Ascospores with crests flat. ×1000

Fig. 4. *A. variecolor*

(*a*) Mature head
(*b*) Conidia
(*c*) Foot-cell
(*d*) Ascospores with two equatorial crests
(*e*) Ascospores with crests flat

Fig. 5. *A. unguis* (Emile-Weil & Graudin) emend Thom & Raper ×1000

(*a*) Mature head
(*b*) Conidia
(*c*) Foot-cell
(*d*) Spicule hyphae

Fig. 6. *A. amstelodami* Mangin emend Thom & Raper ×1000

(*a*) Mature head
(*b*) Conidia of various shapes
(*c*) Foot-cells
(*d*) Ascus
(*e*) Ascospores with equatorial furrow

XANTHOMONAS UPPALII SP. NOV. PATHOGENIC ON IPOMOEA MURICATA

By M. K. PATEL

(Accepted for publication June 10, 1948)

IN the rainy season of 1947, a few plants of the creeper, *Ipomœa muricata* R. & Sch. growing on the hedges of fields and the banks of the river near Poona, were found to be suffering from wilt. The symptoms of the disease resembled so closely those shown by the French bean plants blighted by *Xanthomonas phaseoli* (E. F. Sm.) Dowson, or cowpea plants, *Vigna catjang* Walp., attacked by *Xanthomonas vignicola* Burkh., that it was at first suspected that the one or the other of the above two organisms may have caused the blight of the *Ipomœa* creeper. Search through literature revealed, however, that no bacterial blight or leaf-spot had ever been reported on any species of the genus *Ipomœa*.

Isolation

The pathogen could very easily be isolated on dilution poured plates with the nutrient agar or potato dextrose agar; on the latter medium, the organism forms yellow, shining, round colonies which after three days go on increasing in size.

Symptoms

The lesions on the leaves first appear as minute spots with bright yellowish area which, when enlarged, dry out and become brown and brittle. The spots vary in size and number and when numerous, may coalesce involving a large part of the leaf. These lesions are generally due to external infection probably through the stomata or may at times develop from the bacteria from the vascular system. In the latter case, infection assumes a 'V' shape. Around these lesions is generally found a golden yellow border, and surrounding this, there is often a narrow pale green zone. Infection sometime follows the veins and when severe, brings about distortion and wilting of the leaves. Once in the vascular system of the plant, the organisms migrate slowly and have often been recovered two inches from the nearest point of infection. The incubation period is generally seven days. So far, no infection has been observed on the stem or the pods.

In general, the infection on *Ipomœa* leaves *viz.*, the spots and the other general symptoms, very much resembles that caused by the French bean or the cowpea blight organisms, *Xanthomonas phaseoli* and *Xanthomonas vignicola* respectively. To see if any one of these bacterial pathogens was responsible for the disease on the creeper, fresh isolations of those pathogens were made. Those isolates and also an isolate from the diseased *Ipomœa* creeper were selected from single colonies and cross inoculated, the *Ipomœa* pathogen on cowpeas and on French beans and *vice versa*. Whereas the *Ipomœa* pathogen was able to infect the creeper, it failed to infect either cowpeas or French beans. Similarly, while cowpea and French bean pathogens could infect their respective hosts, they were unable to infect the *Ipomœa* creeper. When it thus became evident that these bacteria are specific in their host reactions, the *Ipomœa* pathogen was further studied as far as morphology, cultural characters, biochemical reactions and pathogenicity were concerned.

Morphology

The bacterium is a rod with rounded ends. In 48 hour-old culture grown on potato dextrose agar at 27°C., the size of the cells is 2.2 (2 to 2.4)μ by 0.9 (0.7 to 1.1)μ. It is motile by a single polar flagellum. It is yellowish, Gram-negative, non-capsulated, not acid fast, without spores or involution forms, single or in pairs but never in chains and stains readily with common dyes.

Cultural characters

On nutrient agar slants, the organism forms in 48 hours a fair, smooth, slightly raised, dull, filiform, opalescent growth with lemon-chrome colour and without distinctive odour. On potato dextrose agar slants, it develops copious, smooth, raised, glistening, filiform, opalescent, butyrous growth with empire yellow colour and without distinctive odour. In nutrient broth, a cloudy growth without a pellicle, sediment and floccules develops in 96 hours. In litmus milk after a week's growth there is complete reduction of litmus with reddish tinge at top with light pellicle and heavy sediment but tyrosine crystals are not present in the medium. In plain milk, the growth is fairly good and a pellicle is present, but sediment, floccules and tyrosine crystals do not occur. On Simmon's citrate medium, the growth is good, lemon yellow in colour, and colonies are circular with entire margins. The pathogen is a strict aerobe. The optimum temperature for its growth is about 30°C; growth ceases at and below 10°C and at 40°C or above. The thermal death point is at about 51°C.

Biochemical reactions

Gelatin liquefaction begins on the second day and proceeds rapidly. Hydrogen sulphide tests made with strips of filter paper impregnated with lead acetate showed positive production after 4 days. The Gore's method shows no indol formation. Nitrites and ammonia are not formed at the end of 2, 4 and 6 days. In Uschinsky's, Cohn's and Koser's uric acid media, the growth is inhibited. In broth containing 3 per cent sodium chloride, the growth of the organisms is retarded while it is completely inhibited by a 4 per cent solution. Acetyl-methyl-carbinol is not produced.

The *Ipomœa* pathogen grows well on several media containing carbohydrates but in none of these was acid or gas produced. It gave moderate to good growth in dextrose, lactose, sucrose, mannitol, raffinose, salicin, galactose and xylose while no growth takes place in levulose, arabinose and rhamnose. Starch is hydrolysed. On potato cylinders, the growth is abundant, yellow, shining, flowing and covering the entire surface in 7 days. The bacterium is able to digest casein and grows on media with pH ranging from 5.3 to 9.2; but the best growth occurs, however, at 7 pH.

Pathogenicity

In repeated trials, it was found that the *Ipomœa* organism is pathogenic only to *Ipomœa muricata* and *Ipomœa* species while it failed to infect *I. batata*, *I. purpurea*, *Phaseolus vulgaris*, *Ph. lunatus*, *Dolichos lablab* and *Vigna catjang*.

The *Ipomœa* pathogen differs definitely from *X. phaseoli* and *X. vignicola* in both pathogenicity and cultural reactions. It does not agree in cultural reactions and its pathogenicity with any other bacterial plant pathogen. Since no bacterial blight or leaf-spot has ever been reported on species of *Ipomœa*, this organism is

considered to be a new species for the reasons given above and it is proposed to name it, *Xanthomonas Uppalii* after Dr. B. N. Uppal, former Plant Pathologist and now the Director of Agriculture, Bombay Province, Poona who brought the plant pathological work of the Province to the forefront.

Xanthomonas Uppalii sp. nov. Rods with rounded ends. 2.2 (2.0 to 2.4)μ ×0.9 (0.7 to 1.0)μ. Motile with a single polar flagellum. Gram-negative. Non-capsulated. Not acid fast. No spores. Mostly single. Gelatin liquefied. Fair, smooth, dull, filiform, lemon chrome on nutrient agar. Litmus in milk reduced. Nitrites and ammonia not formed. Indol not produced. Hydrogen sulphide produced. No growth in Ushinsky's, Cohn's and Koser's uric acid media. Acetyl-methyl-carbinol not produced. Good growth with no acid and gas in dextrose, lactose, sucrose, mannitol, raffinose, salicin and xylose while levulose, arabinose and rhamnose not utilised. Starch hydrolysed. Strict aerobe. Optimum temperature for growth 30°C. Thermal death point about 51°C. Pathogenic to *Ipomœa muricata*.

Culture deposited in the Indian Culture Collection of Fungi at the Indian Agricultural Research Institute, New Delhi, and at the Commonwealth Mycological Institute, Kew, England.

The author wishes to express his thanks to Dr. B. B. Mundkur for his help while writing this article.

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A COMPARATIVE STUDY OF SOME WILT-PRODUCING PHYTOPATHOGENIC BACTERIA [1]

By V. P. BHIDE

(Accepted for publication June 8, 1948)

AMONG the phytopathogenic bacteria, nine species are known to cause vascular necrosis. These vascular parasites do not constitute a taxonomic group like the soft-rot, green-fluorescent, and gall-forming organisms but are taxonomically very heterogeneous. In their cultural reactions they can be separated into two groups that have several reactions in common. These organisms are as follows: *Xanthomonas campestris* in cabbage, *X. lespedezæ* in species of Lespedeza, *Corynebacterium flaccumfaciens* in common beans, *C. michiganensis* in tomatoes, *C. sepedonicum* in Irish potatoes, *C. insidiosum* in alfalfa, *Erwinia tracheiphila* in cucumbers, *Pseudomonas solanacearum* in members of the Solanaceæ, and *Bacterium stewartii* in maize.

No attempt has so far been made to study these organisms comparatively as two groups of vascular parasites, although some of them have been studied culturally in detail. Therefore it was hoped that a comparative study of their growth response might shed some new light on their one common characteristic, their host-parasite relationship. The present study comprises two phases of the activity of these bacteria, namely their growth response and parasitism.

SOURCE OF CULTURES

The cultures of the pathogens used in the present study (32 in number) were obtained from various workers in the U. S. A. A type culture of each species was obtained from the American Type Culture Collection in Washington, D. C. Three or more isolates of each organism were obtained except *Corynebacterium sepedonicum* and *Erwinia tracheiphila* where one isolate of each species was used. Before the study was begun, the cultures were tested for their purity and pathogenicity, and maintained on slants of nutrient dextrose agar.

GROWTH REACTION AND CULTURAL RESPONSE OF THE WILT BACTERIA

Morphology and Staining Reactions

The morphological characters of the bacterial species studied are well established and therefore no attempt was made to make a detailed study. All the organisms were rod shaped, did not form any spores, and were not acid fast.

Motility

All the isolates of *Pseudomonas solanacearum*, *Xanthomonas campestris*, *Xanthomonas lespedezae*, *Corynebacterium flaccumfaciens*, and *Erwinia tracheiphila* were motile, whereas the remaining species were non-motile when grown on semisolid agar as recommended in the Manual of Methods (1936).

[1] Condensed from a thesis (No. 886), submitted to the Graduate Faculty of the Iowa State College of Agriculture and Mechanic Arts, Ames, Iowa, U.S.A., in partial fulfillment for the degree of Doctor of Philosophy. Two type-written copies of the complete thesis are on file at the Library of the Iowa State College.

Gram's stain

Both young and old cultures of all the isolates were stained by the Hucker modification of Gram's stain. All the *Corynebacteria* were Gram positive whereas the rest of the species were Gram negative. Smears of *Bacillus subtilis* stained at the same time were Gram positive.

CULTURAL CHARACTERS

The cutural characters of the organisms were studied on various media prepared according to standard methods. The cultures were incubated at 25° C unless otherwise mentioned. The media employed were: nutrient agar, nutrient broth, nutrient dextrose agar, potato dextrose agar, and potato plug. The colony characters were studied on plates of nutrient dextrose agar.

Growth was poor on nutrient agar and nutrient broth; nutrient dextrose agar and potato dextrose agar were very suitable for growth of all the species. Potato plugs were also suitable and the organisms produced bright colours on this medium.

In general, the predominent colour of all the species was some shade of yellow on all the media. *Corynebacterium sepedonicum* and *Erwinia tracheiphila* were white and *C. insidiosum* produced blue-black granules. *Pseudomonas solanacearum* was dirty white, turning brown on all solid media, especially potato plug.

Colonies of almost all the isolates on nutrient dextrose agar plates were round, with entire margins, and without particular markings. Seven day old colonies ranged in diameter from 10 to 12 mm. in the two *Xanthomonas* species and to two to three mm. in *Erwinia tracheiphila*. The rest of the species were intermediate.

THE INFLUENCE OF TEMPERATURE ON GROWTH

The effect of temperature on growth of the organisms was studied on slants of nutrient dextrose agar incubated at various temperatures. Duplicate cultures were carried at 15°, 20°, 25°, 30°, 37°, and 40° C respectively and growth was measured in an arbitrary manner at the end of one, three and five days.

The results showed that *Xanthomonas campestris*, *X. lespedezae*, *Pseudomonas solanacearum*, and *Bacterium stewartii* have a wide growth temperature range of 15° to 40° C. Of the species of *Corynebacterium*, *C. flaccumfaciens* seems to be the only one that has a wide range of temperature for growth, while the rest of the *Corynebacteria* have very narrow limits, the optimum being around 25°C which is also true for *Erwinia tracheiphila*.

GROSS PHYSIOLOGICAL CHARACTERS

The gross physiological characters were studied using standard methods with slight modifications in some cases. The cultures were incubated at 25°C. Table 1 summarises the results.

Liquefaction of gelatin

The ability of the organisms to liquefy gelatin was determined by growing them on three gelatin media, namely plain gelatin, nutrient gelatin, and Frazier's gelatin medium. The results showed that *Xanthomonas campestris* and *Xanthomonas espedezae* are strong gelatin attackers and can even utilise plain gelatin. Of the

species of *Corynebacterium*, *Corynebacterium flaccumfaciens* and *Corynebacterium michiganensis* attacked gelatin moderately. The different isolates of these two species varied among themselves in this respect. All the rest of the species of wilt bacteria did not attack gelatin.

Hydrolysis of starch

The production of amylase in culture was studied on plates of nutrient agar containing 0.5 per cent potato starch. Hydrolysis of starch was tested after five days incubation by flooding the plates with a weak solution of iodine. A clear zone around the colony indicated starch digestion. The results showed that *Xanthomonas campestris* and *Xanthomonas lespedezæ* were very strong starch digestors and *Corynebacterium sepedonicum* attacked it moderately. The rest of the organisms had no action on starch.

Production of hydrogen sulphide

Two methods are generally employed for the detection of hydrogen sulphide in bacterial cultures. Some workers have used solid media containing salts of iron, lead, or cobalt as indicators whilst others have employed liquid media over which lead acetate or lead carbonate impregnated strips of filter paper are suspended. In the present work both the methods were employed. The solid media used were Kligler's iron agar and the medium described by Vaughn and Levine (1936). None of the species produced any hydrogen sulphide on either of these two media. The liquid media used were nutrient broth, nutrient broth containing cystine, and nutrient agar. Only two species, *Xanthomonas campestris* and *Xanthomonas lespedezæ* produced hydrogen sulphide in nutrient broth as evidenced by the darkening of the filter paper strips (impregnated with lead acetate) that were hung over the cultures. On the other two media all the species except *Pseudomonas solanacearum* produced hydrogen sulphide in one week.

Of the species under study, positive hydrogen sulphide production is reported for *Xanthomonas campestris* and *Xanthomonas lespedezæ*, while *Corynebacterium sepedonicum* and *Erwinia tracheiphila* are reported as feeble producers of hydrogen sulphide (Bergey *et al*, 1939). The rest of the wilt bacteria are reported as non-producers of hydrogen sulphide. In the present study the only species that failed to produce it was *Pseudomonas solanacearum*. It seems that the production of hydrogen sulphide is a common character of most of the wilt bacteria.

Production of indole

The ability of the organisms to produce indole was tested by growing them on nutrient broth containing 0.01 per cent tryptophane. Duplicate cultures were incubated and the presence of indole was tested at the end of five and ten days with Kovacs' reagent. An indole positive strain of *Escherichia coli* was used as control. None of the cultures showed presence of indole at the end of five days whilst two cultures of *Xanthomonas campestris* and all the cultures of *Xanthomonas lespedezæ* gave a positive indole test at the end of 10 days. *Bacterium stewartii* reported as a feeble producer of indole did not give a positive test after 10 days growth.

Action on litmus milk

Duplicate cultures in litmus milk were incubated at 25°C. and observations were made at the end of 3, 5, 10, 15, and 30 days. A definite acid reaction was

produced by all cultures of *Corynebacterium insidiosum* but no coagulation was noticed; moderate reduction of litmus was evident after 15 days. *Pseudomonas solanacearum* produced a definite alkaline reaction. *Erwinia tracheiphila* produced no change in the milk. All cultures of *Xanthomonas campestris* and *Xanthomonas lespedezæ* showed complete proteolysis in 15 days. *Corynebacterium flaccumfaciens* also produced moderate proteolysis. *Corynebacterium michiganensis* curdled the milk and reduced the litmus moderately; *Corynebacterium sepedonicum* showed slight reduction but no other change.

Reduction of nitrates

Reduction of nitrates was tested on two media, namely nutrient broth containing 0.1 per cent potassium nitrate and the synthetic semi solid nitrate medium recommended by Zobell (1932). On the former medium, *Pseudomonas solanacearum* alone gave a positive test for nitrites at the end of five days when tested with sulfanilic acid and *L-napthylamine*. Nitrates were present in cultures of the rest of the species when tested with zinc dust. On the synthetic nitrate medium, the *Corynebacteria* and *Erwinia tracheiphila* failed to grow, whereas rom the rest of the species, *Pseudomonas solanacearum* alone reduced nitrates.

Since *Pseudomonas solanacearum* was the only organism that reduced nitrates, it was thought advisable to find out whether this species reduced nitrates beyond the nitrite stage. Tubes of synthetic nitrate medium were therefore inoculated with this organism and the cultures were tested for the presence of nitrites and nitrates at the end of 3, 8, 10, 15, 20, and 30 days. Nitrites and nitrates were both present in 15 days old cultures but both disappeared after 20 days. It seems therefore that this species can utilise nitrites in addition to nitrates.

The Voges-Proskauer and Methyl-Red tests

The ability of the organisms to produce acetyl-methyl-carbionol (V-P test) and produce enough acidity to give a positive reaction with methyl red, was tested by growing the organisms in Difco-MRVP medium. All the cultures were negative for both these tests.

Citrate utilisation test

The organisms were grown on Koser's citrate medium for five days to find out whether any of the species could utilise sodium citrate as the sole source of carbon. Of the species under study, *Xanthomonas campestris*, *Xanthomonas lespedezæ*, and *Pseudomonas solanacearum* alone grew on this medium and produced heavy turbidity. The rest of the species failed to grow on this medium.

Growth on synthetic asparagine medium

The organisms were grown on the synthetic asparagine medium of Starr and Weiss (1943) using the serial transfer technique of these workers. Only *Pseudomonas solanacearum* and *Bacterium stewartii* grew on this medium whereas the two species of *Xanthomonas* and all the *Corynebacteria* failed to grow on it. *Erwinia tracheiphila* was not used in this test as a culture of it was not available at that time. These results are in agreement with those of Starr and Weiss (*ibid*) that only the green-fluorescent plant pathogens of the genus *Pseudomonas* and *Bacterium stewartii* can utilise asparagine as a source of carbon and nitrogen.

In another test, one per cent dextrose was added to the asparagine medium as an additional source of carbon and inoculations of all the species were made on plates of this medium., *Xanthomonas campestris*, *Xanthomonas lespedezæ*, *Bacterium stewartii* and *Pseudomonas solanacearum* showed positive growth on this medium but the *Corynebacteria* failed to grow. It seems therefore that the two species of *Xanthomonas* used in this study can utilise asparagine as a source of nitrogen but not of carbon. These results agree with those of Starr (1946).

UTILISATION OF CARBON COMPOUNDS

In the present work, the fermentation of dextrose was first studied in a preliminary manner by growing the organisms in nutrient broth containing one per cent dextrose in Durham fermentation tubes. Brom-thymol-blue was used as an acid-base indicator. The cultures were incubated at 25°C. and observations were recorded on alternate days.

None of the species under study produced any gas in two weeks. *Xanthomonas campestris*, *Xanthomonas lespededezæ*, and *Pseudomonas solanacearum* produced an alkaline reaction which persisted. All the four species of *Corynebacterium*, *Bacterium stewartii*, and *Erwinia tracheiphila* produced an acid reaction.

As none of the species produced any gas, it was thought unnecessary to use a liquid medium in fermentation tubes. Nutrient agar was therefore used in subsequent trials. The medium was made neutral to brom-thymol-blue which was added to it at the rate of three ccs. of a 1.6 per cent alcoholic solution to a litre of the medium. This large amount of indicator was used to obtain sharp colour changes in the medium. The different carbohydrates were added to this medium at the rate of one per cent and the medium was used in slants. Inoculations were made by streaking the slope of the slant and also stabbing the butt with a straight needle. This procedure, however, proved unnecessary as none of the organisms grew anaerobically in the butt of the slant which showed no colour changes. The cultures were incubated for one month in every case. Since nutrient broth was used as a basal medium, positive utilisation of the fermentable substance was indicated by production of acid. An alkaline reaction indicated that either no acid was produced or if produced, it was neutralised by the ammonia resulting from the breakdown of the peptone. The following carbon compounds were tested: arabinose, xylose, dextrose, maltose, cellobiose, lactose, sucrose, melezitose, pectin, starch, inulin, esculin, salicin, glycerol, mannitol and dulcitol.

Xanthomonas campestris, *Xanthomonas lespadezæ*, and *Pseudomonas solanacearum* did not produce acid from any of the carbon compounds tested, but produced large amounts of alkali. All the four species of *Corynebacterium* produced acid from majority of the carbon compounds tested, except the pentoses and starch. The only exception was *C. sepedonicum* which did not produce any acid from maltose and gave a distinct alkaline reaction with glycerol, mannitol, and dulcitol; *Bacterium stewartii* produced acid from all the test substances and further utilised the acid as evidenced by a reversion to neutrality of the medium. *Erwinia tracheiphila* produced acid from the pentoses, hexoses, disaccharides, trisaccarides and pectin but not from any of the other compounds.

Since *Xanthomonas campestris*, *Xanthomonas lespedezae*, *Pseudomonas solanacearum*, and *Bacterium stewartii* could grow on an inorganic salt medium and since the first three species did not produce any acid from carbohydrates in a peptone

Table I

Gross physiological reactions of the wilt bacteria

Organism	Gelatin lique.	Litmus milk	Indole	Starch hydro	H_2S	NO_3 red.	Citrate Util.	Util inorg. N	Growth temp. range °C
Group I.									
Xanthomonas campestris ..	+	proteo.	+	+	+	−	+	+	15–40
Xanthomonas lespendezæ ..	+	proteo.	+	+	+	−	+	+	15–40
Bacterium stewartii ..	−	no action	−	−	+ weak	−	−	+	15–40
Pseudomonas solanacearum ..	−	alkali	−	−	−	+	+	+	20–40
Group II.									
Corynebacterium flaccumfaciens ..	−	proteo.	−	−	+	−	−	−	15–40
Corynebacterium michiganensis ..	+	curd.	−	−	+	−	−	−	20–30
Corynebacterium sepedonicum ..	−	reduct.	−	+ weak	+	−	−	−	15–25
Corynebacterium insidiosum ..	−	reduct.	−	−	+	−	−	−	15–25

basal medium, it was thought worthwhile to employ an inorganic nitrogen basal medium to study the utilisation of carbon compounds by these species. The medium used was a modification of the one recommended by Ayers, Rupp and Johnson (1919). The composition of this medium was as follows :

$(NH_4)_2PO_4$	1.0 gm.	Agar	15.0 gm.
KCl	0.2 ,,	Water	1.0 litre
$MgSO_4 . 7H_2O$	0 2 ,,	Carbon compound ..	10.0 gm.
$CaCl_2$	0.2 ,,		

The calcium chloride was dissolved separately and added to the bulk of the medium to prevent precipitation. The medium was made neutral to brom-thymol-blue and plates were poured. In addition to all the carbon compounds tested previously, formic, acetic, lactic, citric, tartaric, salicylic, oxalic and benzoic acids and ethyl alcohol were also tested. In the case of the organic acids, the reaction of the medium was readjusted with 0.1 N NaOH after the addition of the acid in each case. Ethyl alcohol was added to the sterile, cooled medium just prior to pouring plates. In every case, the various isolates of the same species were inoculated in the same plate which was divided into sectors. Inoculations were made with a standard platinum loop from young broth cultures. The plates were incubated at room temperature and observations were made at the end of five and ten days. Growth was recorded as negative only when it was not evident after 15 days incubation.

As the only source of energy in every case was the particular carbon compound added, utilisation of this substance was evidenced by growth of the organism. The results are recorded in Table II.

The results show that the two species of *Xanthomonas* can utilise a large number of carbon compounds and are very much alike in this respect. The only exception is mannitol which was utilised by the cabbage but not the *Lespedezæ* organism. Of the organic acids tested, only acetic and citric acids can support growth of these two species.

Pseudomonas solanacearum on the other hand can utilise a very limited number of carbon sources. It differs from the *Xanthomonas* species in its inability to utilise xylose, maltose, lactose, melezitose, starch, esculin, and acetic acid but it can utilise glycerol.

Bacterium stewartii differs from both *Xanthomonas* and *Pseudomonas* in its ability to utilise arabinose but not cellobiose and citric acid.

UTILISATION OF ORGANIC NITROGEN

It has been pointed out previously that the species of *Corynebacterium* under study failed to grow in synthetic media containing potassium nitrate or asparagine as the source of nitrogen. It would seem therefore that these species require complex source of nitrogen.

Mushin (1938) studied the food requirements of *Pseudomonas solanacearum* and *Corynebacterium michiganensis*, both causing vascular necrosis in tomato. She grew these two pathogens on synthetic media containing different sources of carbon and nitrogen that are likely to be found in the vascular system of the tomato plant and found that asparagine, peptone, tyrosine, and glutamic acid served both as

Table II

Utilization of carbon compounds employing ammonium phosphate as source of nitrogen.

Organism	No. of isolates	Arabinose	Xylose	Dextrose	Maltose	Cellobiose	Lactose	Sucrose	Melezitose	Pectin	Starch	Inulin	Esculin	Salicin	Glycerol	Mannitol	Dulcitol	Ethyl alc.	Organic acids: Formic	Acetic	Lactic	Citric	Tartaric	Salicylic	Oxalic	Benzoic
Xanthomonas campestris	5	−	+	+	+	+	+	+	+	+	+	−	+	−	−	+	−	−	−	+	−	+	−	−	−	−
Xanthomonas hespedeæ	3	−	+	+	+	+	+	+	+	+	+	−	+	−	−	−	−	−	−	+	−	+	−	−	−	−
Bacterium stewartii ..	5	+	−	+	+	−	+	+	−	+	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−
Pseudemonas solanacearum	3	−	−	+	−	+	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−

sources of carbon and nitrogen for *Pseudomonas solanacearum*. *Corynebacterium michiganensis*, on the other hand, could only grow on a medium containing peptone as the source of carbon and nitrogen. Stapp (1930) found that only some proteins and amino acids could serve as sources of carbon and nitrogen for *Corynebacterium michiganensis*. The nitrogen requirements of other wilt bacteria have not yet been worked out.

In the present study the organisms were grown on a basal inorganic medium to which various organic nitrogen compounds (mainly amino acids) were added as sources of nitrogen and carbon. In one series, no dextrose was added to the medium whereas in another series of cultures, one per cent dextrose served as a source of carbon. The basal medium was the same as the one used by Mushin (1938) and consisted of :

K_2HPO_4	3.1 gm.
KH_2PO_4	0.8 gm.
KCl	0.2 gm.
$MgSO_4$	0.20 gm.
Water	1.0 litre

In the preliminary experiments, a medium solidified by the addition of 1·5 per cent washed, purified Bacto agar was used. The pH of the medium was adjusted to neutrality to brom-thymol-blue, which was incorporated in the medium at the rate of 1 cc. of 1·6 per cent alcoholic solution to a litre. The amino acids used were glycine, β-alanine, l-leucine, l-tyrosine, l-tryptophane, l-cystine, d-lysine, d-arginine, l-aspartic acid, d-glutamic acid, creatine, creatinine, and d-l-isoleucine. In addition to these, choline, sarcosine, and para-aminobenzoic acid were also tested. In each case 0·2 per cent of the test substance was used except tryptophane and arginine, where only 0·1 per cent was used. Proteose peptone was used as check. The reaction of the medium had to be readjusted in some cases with 0·1 N NaOH. Cystine was dissolved in dilute hydrochloric acid before addition to the medium.

Plates were poured in each case and divided into eight sections on the bottom of each plate with a glass marking pencil. Each sector was inoculated with one of the eight organisms under study ; *Erwinia tracheiphila* was not included as a culture of it was not on hand at the time of these trials. Inoculations were made with a straight needle from broth cultures of proved pathogenicity. The plates were incubated at 25° C and observations were made after a week.

Whenever growth was obtained, the results were confirmed by reinoculation in tubes of a liquid medium of the same composition as the solid one. The serial transfer technique described by Starr and Weiss (1943) was used for these inoculations. The results of this experiment are recorded in Table III.

TABLE III

Utilization of organic nitrogen with and without dextrose as source of carbon

Source of nitrogen.	*Xanthomonas campestris*		*Xanthomonas lespedezae*		*Pseudomonas solanacearum*		*Bacterium stewartii*		*Corynebacterium flaccumfaciens*		*Corynebacterium michiganesis*		*Corynebacterium insidiosum*		*Corynebacterium sepedonicum*	
	with dextrose	without dex.	with dextrose	without dex.	with dextrose	without dex.	with dextrose	without dex.	with dextrose	without dex.	with dextrose	without dex.	with dextrose	without dex.	with dextrose	without dex.
Glycine	−	−	+	−	+	−	+	−	−	−	−	−	−	−	+	−
β-alanine	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−
Leucine	+	−	+	−	+	−	+	−	+	−	+	−	−	−	−	−
Isoleucine	+	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−
Tyrosine	+	−	+	−	+	+	+	−	+	−	+	−	−	−	+	−
Tryptophane	+	−	+	−	+	−	+	−	−	−	−	−	−	−	−	−
Cystine	+	+	+	−	+	−	+	−	+	−	+	−	−	−	−	−
Lysine	+	+	+	−	+	−	+	−	+	−	+	−	−	−	+	−
Arginine	−	−	−	−	+	−	+	−	+	−	+	−	−	−	−	−
Aspartic acid	+	+	+	−	+	+	+	−	+	−	+	−	−	−	−	−
Glutamic acid	+	+	+	+	+	+	+	+	+	−	+	−	+	−	+	−
Creatine	+	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−
Creatinine	+	−	+	−	+	−	+	−	−	−	−	−	−	−	+	−
Choline	+	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−
Para-aminobenzoic acid	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
Sarcosine	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−
Proteose peptone (check)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

The results show that *Corynebacteria* are very inactive in utilisation of amino nitrogen ; *Corynebacteria insidiosum* was the most inactive species in this group. None of these organisms could utilise the substances tested as sole sources of carbon and nitrogen.

Of all the substances tested glutamic acid, in the presence of dextrose, supported growth of all the species tested. In the absence of dextrose as a source of carbon, it could support the growth of *Xanthomonas campestris*, *Xanthomonas lespedezae*, *Pseudomonas solanacearum* and *Bacterium stewartii*. Some striking differences were shown by *Xanthomonas* species in their utilisation of amino nitrogen ; *Xanthomonas campestris* could utilise cystine, lysine, and aspartic acid as sources of both carbon and nitrogen whereas *Xanthomonas lespedezæ* could not.

Pseudomonas solanacearum could utilise β-alanine, tyrosine, and aspartic acid as sole sources of carbon and nitrogen.

It is probable that these results may have a bearing on the pathogenicity and virulence of the organisms studied.

HOST RELATIONS OF THE WILT BACTERIA

An attempt has been made in Table IV to summarise information regarding the pathogenesis of the wilt pathogens under study; this information has been compiled from the existing literature on the subject.

MODE OF HOST INVASION

Invasion through wounds

An examination of Table IV shows that the most common method of host invasion is through wounds, although some of the pathogensare able to invade the host through natural openings such as stomata and hydathodes.

The type of wounds is diverse. *Corynebacterium michiganensis* and *Corynebacterium flaccumfaciens*, which are seed borne, enter the host through wounds on the cotyledons and young leaves.

TABLE IV

Comparative pathogenesis of nine bacterial wilt organisms

Organism	Common host	Mode of host invasion	Host tissue invaded	Mode of perpetuation
Xanthomonas campestris	crucifers	hydathodes, wounds, stomata	xylem	on seed; in soil
Xanthomonas lespedezæ	annual lespedezæ	wounds ; stomata	xylem	in or on seed

Table IV—*concld.*

Organism	Common host	Mode of host invasion	Host tissue invaded	Mode of perpetuation
Corynebacterium flaccumfaciens	common bean	wounds	xylem	in seed
Corynebacterium michiganensis	tomato	wounds ; stomata	phloem	on seed
Corynebacterium sepedonicum	potato	wounds ; root tips	xylem	in seed tubers
Corynebacterium insidiosum	alfalfa	wounds	xylem	on seed; in soil
Bacterium stewartii	sweet corn	wounds ; hydathodes	xylem	on seed; in soil ; in insects
Pseudomonas solanacearum	members of Solanaceae	wounds	xylem	in soil
Erwinia tracheiphila	cucumber	wounds	xylem	in cucumber beetles

On the other hand, *Pseudomonas solanacearum* and *Bacterium stewartii* enter their respective hosts through wounds on the roots. The cucumber beetle which feeds on leaves is responsible for transmitting *Erwinia tracheiphila*. Wounds caused during transplanting and topping tomatoes provide a means of entry for *Corynebacterium michiganensis*.

Of the nine wilt pathogens under study, three, namely *Corynebacterium flaccumfaciens*, *Corynebacterium insidiosum*, and *Pseudomonas solanacearum* have not been shown yet to invade their hosts in any other manner except through wounds. Of these three species, the two *Corynebacteria* are carried on the seed whereas *Pseudomonas solanacearum* seems to be the only wilt pathogen that persists in the soil primarily.

Invasion through stomata and hydathodes

The only wilt pathogen that normally invades its host through natural openings is *Xanthomonas campestris*. Entry in this case is through hydathodes situated on the leaves at the terminations of veins. Smith (1911) records invasion of maize by *Bacterium stewartii* through hydathodes but this is not normal in Stewart's wilt.

Stomatal invasion has been reported in the case of *Xanthomonas campestris* (Drechsler, 1919), *Xanthomonas lespedezæ* (Ayers *et al*, 1939), and *Corynebacterium michiganensis* (Bryan, 1930).

Infection of potato by *Corynebacterium sepedonicum* through unwounded root tips has recently been reported by Tyner (1946) in greenhouse experiments.

It seems, therefore, that *Xanthomonas campestris* is the only wilt pathogen that is able to invade the host and spread in it as a result of invasion through natural openings.

Host tissue invaded

The wilt bacteria in all cases primarily cause vascular necrosis which results in wilting of the host plant. Xylem is invaded in all cases except in tomato canker caused by *Corynebacterium michiganensis*. This organism invades the phloem and also comes out on the surface of the host causing cankerous patches. *Pseudomonas solanacearum* is another parenchymo-vascular organism. The rest of the wilt bacteria strictly invade the xylem and never come out on the host surface. In most cases, however, the parenchyma surrounding the xylem vessels is destroyed, but the bacteria are not occluded in the parenchyma before the vessels are completely filled by them.

The movement of the wilt bacteria inside their hosts varies with the different organisms. In cabbage and *Lespedezae* wilts, the bacteria usually gain entrance through the leaves and then move downwards in the stem. Bacteria have not been found in the root system in *Lespedezae* wilt and this is probably due to the death of affected plants before the bacteria have reached the roots ; this is not the case in cabbage where wilting is more gradual.

In Stewart's wilt of maize, the movement of the bacteria is usually upwards and they are also found in the root system in later stages of the disease. Root infestation is, of course, evident when infection occurs through roots. In the case of *Pseudomonas solanacearum*, infection through the roots is the rule, and the movement of the organisms is always upwards ; in artificial inoculations, the bacteria can travel both upwards and downwards. A similar phenomenon occurs in wilt of cucumbers where the organism enters into the aerial parts of the plant through the bites of the cucumber beetle.

The *Corynebacteria* form a group of wilt bacteria by themselves. As far as is known, movement is generally upwards except in *Corynebacteria michiganensis*.

HOST RANGE

Review of literature

The natural hosts of the wilt bacteria are mostly annuals and comprise members of the families Cruciferae, Leguminosae, Solanaceae, Gramineae, and Cucurbitaceae. An attempt is made below to review the existing literature on the host range of the wilt bacteria.

Xanthomonas campestris.—This species was first reported by Pammel (1895) on rutabaga. Elliot (1930) lists the following hosts of the organism : *Brassica arvensis, Brassica campestris, Brassica chinensis, Brassica napus, Brassica nigra,*

Brassica oleracea-acephala, *Brassica oleracea-botrytis*, *Brassica oleracea-capitata*, *Brassica oleracea-caulo-rapa*, *Brassica pekinensis*, *Brassica rapa*, *Matthiola incana*, and *Raphanus sativus*. The susceptibility of stocks (*Matthiola incana*) has been questionable.

Xanthomonas lespedeza.—Ayers, Lefebvre and Johnson (1939) who described this species, record *Lespedeza stipulacea* and *Lespedeza striata* as very susceptible to wilt in the field. Greenhouse inoculations showed that *Lespedeza capitata*, *Lespedeza daurica*, *Lespedeza frutescens*, *Lespedeza inochanica*, *Lespedeza procumbens*, *Lespedeza sericea*, and *Lespedeza virginica* were susceptible. The organism was able to produce small, necrotic areas on leaves of *Melilotus alba* inoculated by means of needle pricks, but the pathogen failed to produce any symptoms on any of the other leguminous plants inoculated in the greenhouse.

Corynebacterium flaccumfaciens—Elliott (1930) records *Phaseolus vulgaris* and *P. lunatus macrocarpus* as the natural hosts of this organism and soybeans were successfully inoculated artificially. Burkholder (1930) obtained infection on *Phaseolus lunatus*, *Phaseolus coccineus*, *Phaseolus angularis*, *Vigna sinensis*, *Vigna sesquipedalis*, *Soja max*, and *Dolichos lablab*.

Corynebacterium michiganensis.—Smith (1911) first reported this organism on potato and he believed that it also occurred on the tomato. Stapp (1930) reported successful infection of *Pisum sativum* and *Phaseolus vulgaris*. Orth (1937) found *Solanum humboldtii* and *Solanum pruniforme* to be susceptible to *Corynebacterium michiganensis*. McNew (1941) found *Hyoscyamus niger* to be a natural host of this organism. In his greenhouse experiments, *Lycopersicum chilensis*, *Nicotiana glutinosa*, and *Nicotiana paniculata* were very susceptible to this organism but potato could not be infected. Ark (1944) reported *Cyphomandra betacea*, *Solanum nigrum* var. *guineense*, and *Nicotiana glutinosa* to be susceptible to this pathogen, but not *Nicotiana tabacum*. *Lycopersicum pimpinellifolium* was slightly susceptible and inoculated plants were never killed.

Corynebacterium sepedonicum.—Till 1914, this organism was reported only on potato. Spieckermann and Kotthoff (1914) reported tomato (*Lycopersicum esculentum*) and *Lycopersicum racimegerum* to be susceptible to this organism but no mention was made of the symptoms produced. Stapp (1930) reported the organism to be mildly pathogenic to tomato. Larson (1944) found that all the commercial varieties of tomato and eggplant (*Solanum melongena*) were highly susceptible to *Corynebacterium sepedonicum;* the wild, scarlet, spiny eggplant (*Solanum integrifolium*) was also equally susceptible.

Corynebacterium insidiosum.—Jones and McCulloch (1926) who described this organism on alfalfa (*Medicago sativa*) reported that no symptoms developed on inoculated plants of red clover and several annual legumes (species not mentioned). Elliott (1930) reports alfalfa and white clover (*Melilotus alba*) as its only hosts.

Bacterium stewartii.—Elliott (1930) reported *Zea mays* as the single host of this organism. Subsequently, *Euchlaena mexicana*, *Coix lachyma-jobi*, *Sorghum vulgare*, and *Euchlaena perennis* were reported as new hosts of this pathogen (Poos and Elliott, 1936 ; Ivanoff, 1935 ; Elliott and Poos, 1940).

Pseudomonas solanacearum.—Of all the wilt bacteria, this species alone has the widest host range extending over several families of flowering plants. Amongst the more important hosts are potato, tomato, eggplant, tobacco, banana, and

peanut. T. E. Smith (1939) carried out extensive field and greenhouse experiments on the host range of this organism and classified the plants tested into three classes on the basis of their susceptibility. These were (*a*) species susceptible to both natural and artificial infection (29 species), (*b*) species susceptible to artificial infection but immune to natural infection (5 species), and (*c*) species immune to both natural and artificial infection (56 species). He also suggested that sweet potato, cotton, watermelon, fireweed, *Crotolaria striata*, velvet bean, lima bean, soybean, and cowpea be removed from the list of susceptible plants and *Xanthium pennsylvanicum*, *Xanthium chinense*, *Physalis pruinosa*, *Aster pilosus*, and *Ambrosia trifida* be added to it.

Erwinia tracheiphila.—Elliott (1930) in her Manual lists *Cucumis melo*, *C. sativus*, *Cucurbita maxima*, *Cucurbita moschata*, and *Cucurbita pepo* as the natural hosts of this organism and *Benincasa cerifera*, *Citrullus vulgaris*, *Cucumis anguria*, *Cucumis odoraissima*, *Cucurbita californica*, *Cucurbita foetidissima*, *Echinocystis lobata*, and *Sicyos angulatus* were successfully inoculated in the greenhouse.

Experimental results

In these experiments the organisms studied were *Xanthomonas campestris*, *Xanthomonas lespedezæ*, *Corynebacterium flaccumfaciens*, *Corynebacterium michiganensis*, and *Corynebacterium insidiosum*. The plants on which inoculations were made were those that had not previously been tested for their susceptibility to one or more of the above organisms. All the plants were grown in four inch pots. Inoculations were made when the plants were about ten days old. The method of inoculation consisted of introducing a water suspension of the bacteria in the stem of each plant by means of a hypodermic syringe. Checks were inoculated with sterile water. The plants were incubated in a moist chamber for 48 hours before and after inoculation. Observations were made at three day intervals and the plants were discarded when a month old. The inoculations were repeated in every case.

Xanthomonas campestris.—The winter stocks (*Matthiola incana*) has been reported as a host of this organism but the evidence on this point is conflicting. In the present experiments, seedlings of this species failed to show any symptoms of wilt in one month when inoculated with *Xanthomonas campestris*; a cabbage seedlings (variety Early Jersey Wakefield) inoculated at the same time developed typical symptoms of wilt and most of them died in a month.

Xanthomonas lespedezæ.—This organism failed to produce any symptoms of wilt in young plants of *Phaseolus lunatus macrocarpus*, *Phaseolus aureus*, *Phaseolus acutifolius*, and *Stizolobium deeringianum* in one month. Seedlings of Korean lespedezae inoculated at the same time, showed typical wilt symptoms in ten days.

Corynebacterium flaccumfaciens.—No symptoms were produced on seedlings of *Phaseolus lunatus macrocarpus*, *Phaseolus aureus*, *Phaseolus acutifolius*, and *Stizolobium deeringianum* whereas plants of Golden Cluster beans inoculated at the same time developed typical leaf symptoms in ten days.

Corynebacterium michiganensis.—The ability of this organism to infect potato or otherwise has not been definitely proved. Young potato plants, a week old, were inoculated with a virulent culture of *Corynebacterium michiganensis*. One set of plants was inoculated by the hypodermic needle method and in another set of plants the tops were cut off with a flamed scalpel and an agar culture of the

organism was applied to the cut ends. No symptoms of wilt appeared in a month's time, whereas tomato seedlings (variety Bonny Best) inoculated at the same time developed typical cankers within ten days.

No infection was produced on young seedlings of *Solanum melongena*, *Capsicum annuum*, and *Physalis pruinosa*.

Corynebacterium insidiosum.—Young seedlings of *Phaseolus lunatus macrocarpus*, *Phaseolus acutifolius*, *Stizolobium deeringianum*, and White, White Dutch, Alsike, Black Medic, Crimson, Red, and Yellow Blossom clovers failed to show any symptoms of infection in one month except in the case of White clover (*Melilotus alba*). Seedlings of alfalfa inoculated at the same time showed typical symptoms of the disease in three weeks.

CROSS INOCULATION TRIALS

The purpose of these trials was to determine whether any of the pathogens under study were able to invade the common hosts of the rest of the species of the wilt bacteria. Cross inoculations were made, therefore, with all the species except *Pseudomonas solanacearum;* this species was not included in these trials as a highly virulent culture of the organism was not on hand at that time.

The plants inoculated were cabbage (Early Jersey Wakefield), Korean lespedeza, beans (Golden Cluster), tomatoes (Bonny Best), potato, alfalfa, sweet corn (Golden Bantam), and cucumbers. The plants inoculated were about two weeks old. A water suspension of bacteria was used in every case and inoculations were made in the stems of the plants with a hypodermic syringe. All the plants were inoculated in a moist chamber for 48 hours before and after inoculation and subsequently transferred to the greenhouse bench.

The cultures of the various pathogens were of proved pathogenicity and had been recently isolated from diseased plants by the author. Table V shows the results.

The test was repeated twice with the same results. It appears from the results that the species of wilt bacteria under study are unable to invade any of the plants tested except their natural hosts with the method and cultures used. The only exception was *Corynebacterium sepedonicum* which could invade tomatoes in addition to its natural host, namely potato.

Wellhausen (1938) reported a certain degree of infection of sweet corn by *Xanthomonas campestris*, *Corynebacterium flaccumfaciens*, *Corynebacterium michiganensis*, and *Corynebacterium insidiosum*. Infection with these organisms was obtained only in very young seedlings of sweet corn. Infection of sweet corn, characteristic of *Bacterium stewartii* was never produced but only leaf symptoms and a little stunting was visible. He also reported *Bacterium stewartii* to be slightly pathogenic to beans but not to tomatoes. In the present experiments *Bacterium stewartii* failed to infect any other plant except sweet corn. Further, none of the other species produced any infection in sweet corn. These conflicting results may be due to the differences in the virulence of the cultures of the pathogen or the technique used.

The ability of *Corynebacterium sepedonicum* to invade tomatoes was confirmed in these trials.

TABLE V.

Results of cross-inoculation trials

Organism	Pathogenicity on							
	Cabbage	Lespedeza	Beans	Tomatoes	Sweet corn.	Cucumbers.	Alfalfa.	Potato.
Xanthomonas campestris	+	—	—	—	—	—	—	—
Xanthomonas lespedezae	—	+	—	—	—	—	—	—
Corynebacterium flaccumfaciens	—	—	+	—	—	—	—	—
Corynebacterium michiganensis	—	—	—	+	—	—	—	—
Corynebacterium sepedonicum	—	—	—	+	—	—	—	+
Corynebacterium insidiosum	—	—	—	—	—	—	+	—
Bacterium stewartii	—	—	—	—	+	—	—	—
Erwinia tracheiphila	—	—	—	—	—	+	—	—

Studies on the host range of any phytopathogenic organism can never be too extensive or "complete" since all that is attempted by any worker is testing of available species, particularly those belonging to the same family as the common host of the organism. Further work, may find other new, unknown hosts of the wilt organisms.

DISCUSSION

The results recorded so far show that the wilt pathogens studied fall into two distinct groups. Group 1, composed of *Xanthomonas campestris*, *Xanthomonas lespedezæ*, *Bacterium stewartii*, and *Pseudomonas solanacearum*, is characterised by the biochemical activity of its members; the cabbage and *Lespedezae* pathogens are especially very active. They hydrolyse starch, liquefy gelatin, proteolyse milk, and produce indole. *Bacterium stewartii* and *Pseudomonas solanacearum* do not possess these characteristics; *Pseudomonas solanacearum* differs from the rest in its inability to produce hydrogen sulphide. In fact it is the only wilt pathogen that has this characteristic.

All the four pathogens in this group multiply rapidly and have a very wide growth temperature range. The pathogens in group 2, on the other hand, grow slowly. Inorganic nitrogen is utilised by all the members of group 1 and they can also utilise organic nitrogen in the form of amino acids.

The members of group 1 seem to be rather primitive organisms when compared with those in group 2, which is composed of the *Corynebacteria*. These pathogens are slow growers, have narrow limits of growth temperatures, and a low biochemical activity in general; gelatin liquefaction is not strong, starch is not hydrolysed usually, indole is not produced, and proteolysis in milk does not occur. *Corynebacterium flaccumfaciens* is more active biochemically than the rest. None of the four *Corynebacteria* in this group can utilise inorganic nitrogen and only a very small number of amino acids are utilised only when sugar is present as a carbon source. It would seem that the nutritive requirements of these organisms are very complex and growth accessory factors may be necessary for their development. This characteristic, coupled with their lack of enzymatic activity, might explain why these organisms are vascular rather than parenchymatous. The biochemical inactivity of these organisms can further be correlated with their pathogenesis; none of the diseases caused by these *Corynebacteria* are as intense in their manifestations as those caused by members of group 1, the active parasites. For example, bean wilt caused by *Corynebacterium flaccumfaciens* and tomato wilt caused by *Corynebacterium michiganensis* are never as destructive as the 'black rot' of cabbage, Stewart's wilt of corn, and the wilt of Solanaceae. It is well known that many plants recover wholly or partially. *Corynebacterium sepedonicum* develops slowly in its host, so much so that the presence of the pathogen in diseased tissues is often difficult to demonstrate.

It is surprising why members of group 1, very active bio-chemically, are vascular parasites. The *Xanthomonas* species are particularly noteworthy in this respect. The genus *Xanthomonas* is made up of species which have the same biochemical activity and majority of the members cause 'leaf-spots' and 'blights'; *Xanthomonas campestris* and *Xanthomonas lespedezæ* are the only exceptions that are vascular parasites. The nutritional requirements of these organisms are very simple and special growth accessory factors are not necessary. Starr (1946) studied the "minimal nutritive requirements" of 30 species of *Xanthomonas* (including *Xanthomonas campestris* and *Xanthomonas lespedezæ*), and found that all the species could grow on a simple basal medium containing ammonium chloride, glucose, and salts and growth accessory factors such as methionine, glutamic acid, and nicotinic acid were not necessary. The only exceptions were *Xanthomonas pruni* which required nicotinic acid to support its growth and *Xanthomonas hederae* and *Xanthomonas transluscens*, which required methionine. Why is it then that in such a homogeneous group of organisms like those in the genus *Xanthomonas*, only a few are vascular parasites? The answer clearly does not lie in the "minimal nutritive requirements" of these organisms, or in their enzymatic activity.

It is interesting to speculate on the existence of antibiotic phenomena in the host relations of the wilt pathogens. In the case of the 'leaf-spot' and 'blight' organisms, it is possible that the bacteria themselves elaborate some chemicals, not necessarily enzymes, which enable them to kill the living cells in the leaf of the host plant, a property that might be absent in the vascular parasites. The existence of such phenomena cannot be demonstrated unless specific microchemical tests are developed and utilised. Before further work is done on the problem, it would

also be worth while to find out whether the species of wilt bacteria are really unit or group species. It is well known that two of the wilt bacteria, namely *Bacterium stewartii* and *Corynebacterium michiganensis* are composed of various strains. Such strains also exist in *Xanthomonas translucens* and the root-nodule organism, *Rhizobium* sp. It is felt that with the development of an appropriate technique for measuring the virulence of these bacteria and by the use of differential hosts, as is done in the case of the rusts, most of the bacterial pathogens could be shown to be very variable and composed of strains. It should prove illuminating to study the growth response of these strains comparatively. In other words, further progress on this problem must combine strain isolations in terms of host reaction and the growth response of the different variants in each species of the wilt bacteria.

SUMMARY

Thirty-two isolates of nine species of wilt producing plant pathogenic bacteria belonging to the genera *Xanthomonas*, *Pseudomonas*, *Corynebacterium*, *Erwinia*, and *Bacterium* were studied comparatively for their cultural behaviour and host relations.

All the species were rod shaped, non-sporulating, non acid-fast organisms. The *Corynebacteria* were Gram positive while the rest were Gram negative. The *Corynebacteria* (with the exception of *Corynebacteria flaccumfaciens*) and *Bacterium stewartii* were non-motile but the rest were motile.

All the isolates produced a yellowish, slimy growth on most of the media used ; exceptions were *Pseudomonas solanacearum* which was dirty-white turning brown, *Corynebacterium sepedonicum*, white and *C. insidiosum*, bluish-black.

Xanthomonas campestris, *Xanthomonas lespedezæ*, *Pseudomonas solanacearum*, *Bacterium stewartii*, and *Corynebacterium flaccumfaciens* had a very wide optimum growth-temperature range of 15°-40° C. ; the rest of the species had narrower limits of 20°-30° C. for optimum growth.

Xanthomonas campestris and *Xanthomonas lespedezæ* were active liquefiers of gelatin and they also hydrolysed starch. *Corynebacterium flaccumfaciens* and *Corynebacterium michiganensis* liquefied gelatin moderately but did not hydrolyse starch, whereas *Corynebacterium sepedonicum* attacked starch moderately but not gelatin. The rest of the species had no action on either.

Hydrogen sulphide was produced by all the species except *Pseudomonas solanacearum*, when tested by the lead acetate strip method. The production of hydrogen sulphide by *Corynebacterium flaccumfaciens*, *Corynebacterium michiganensis*, *Corynebacterium insidiosum*, and *Bacterium stewartii* was demonstrated for the first time.

Only some isolates of *Xanthomonas campestris* and all the isolates of *X. lespedezæ* produced indole weakly.

The two *Xanthomonas* species and *Corynebacterium flaccumfaciens* proteolysed milk; *C. insidiosum* and *Bacterium stewartii* produced slight acidity while *Erwinia tracheiphila* produced no change ; *C. michiganensis* curdled it and *Pseudomonas solanacearum* produced distinct alkalinity.

Psedomonas solanacearum was the only species that reduced nitrate to nitrite and it further utilised nitrite ; the rest of the species did not reduce nitrate.

All the species were Voges-Proskauer and Methyl Red negative. The two *Xanthomonas* species and *Pseudomonas solanacearum* utilised citrate.

Only *Bacterium stewartii* and *Pseudomonas solancearum* could utilise asparagin as the sole source of carbon and nitrogen.

The *Corynebacteria* produced an acid reaction with all the carbon compounds tested when nutrient broth was used as a basal medium. They failed to grow on a synthetic, inorganic nitrogen media with carbon compounds as the source of energy. The *Xanthomonas* species and *Pseudomonas solanacearum* produced an alkaline reaction with all the carbon compounds tested in a peptone basal medium; *Bacterium stewartii* produced an acid reaction which reverted to neutrality with age.

In a synthetic, inorganic nitrogen medium, *Xanthomonas campestris* and *Xanthomonas lespedezæ* utilised a large number of carbon compounds including citric and acetic acids. *Pseudomonas solanacearum* utilised only a small number of these compounds, while *Bacterium stewartii* was intermediate between the *Xanthomonas* species and *Pseudomonas solanacearum* in this respect.

Very striking differences were exhibited by the wilt bacteria in the utilisation of amino acids as sources of nitrogen and or carbon. The *Corynebacteria* were very inert in this respect; glutamic acid was used as a source of nitrogen by all the species in the presence of dextrose as a carbon source.

Wounds are necessary for host invasion by the pathogens in a majority of cases. Majority of the pathogens invade the xylem principally; exception is *Corynebacterium michiganensis* which invades the phloem and causes cankers on the stem.

Winter stock (*Matthiola ancana*) was not susceptible to the strains of *Xanthomonas campestris* used in this study. *Corynebacterium michiganensis* failed to produce any symptoms in potatoes.

In cross-inoculation trials where all the common hosts of the wilt bacteria (except *Pseudomonas solanacearum*) were used, the pathogens did not produce symptoms in any plants except in their common hosts; the only exception was *Corynebacterium sepedonicum*, which could invade the tomato and the potato.

The present study indicates that the wilt bacteria can be divided into two groups based on their differential growth response, namely their enzymatic activity, nitrogen utilisation, and growth temperature range. Definitely these bacterial plant pathogens are not as heterogenous in their growth reaction as their systematic grouping suggests. Group 1 comprises of four species as follows: *Xanthomonas campestris*, *Xanthomonas lespedezæ*, *Pseudomonas solanacearum*, and *Bacterium stewartii*, and Group 2 four also, namely, *Corynebacterium flaccumfaciens*, *Corynebacterium michiganensis*, *Corynebacterium sepedonicum*, and *Corynebacterium insidiosum*. The growth reaction of Group 2 shows considerable specificity on the nitrogen containing amino acids which suggests that the growth of these bacteria in the host is dependent on specific nutrilites elaborated by the interaction of the host and parasite. What these may be can only be determined by strain isolation based on differential host reaction and an intensive study of their growth response.

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INDIAN PHYTOPATHOLOGICAL SOCIETY

Minutes of the Annual meeting held on 2-1-1948 at 3 P.M. at Patna.

Present

Members	*Visitors*
Mr. S. P. Raychaudhuri	Dr. J. J. Chinoy
Mr. S. Y. Padmanabhan	Mr. R. Seshagiri Rao
Dr. B. B. Mundkur	Mr. D. D. Awasthi
Mr. R. C. Lacy	Mr. T. R. Virendra
R. B. J. C. Luthra	
Dr. R. K. Saksena	
Dr. R. S. Vasudeva	
Dr. R. L. Nirula	

1. Dr. B. B. Mundkur explained that in 1947, there was no council, as the members were being enrolled. He was the only officer of the Society, *viz.*, Secretary-Treasurer. He said that the first business was to elect a chairman for the meeting.

2. Rai Bahadur J. C. Luthra was unanimously elected chairman and he took the chair.

3. The Secretary then read his annual report. This was unanimously adopted.

4. The final ballots were then opened and counted by Dr. R. K. Saksena and Mr. S. P. Raychaudhuri. The following were elected office-bearers for 1948.

President	Mr. J. F. Dastur
Vice-President	Dr. S. R. Bose
Secretary-Treasurer	Dr. B. B. Mundkur, 1948 to 1951. Three Years

Councillors

Northern Zone	Dr. Pushkarnath
Delhi Zone	Dr. R. S. Vasudeva
Eastern Zone	Dr. K. C. Mehta
Western Zone	Dr. B. N. Uppal
Central Zone	Mr. G. S. Kulkarni
Southern Zone	Mr. K. M. Thomas

5. The minutes of the meeting held on 28-2-47 were read and confirmed.

6. Art. 5 of the constitution was amended unanimously, after some discussion, as follows :—

Art. 5 (1) All persons interested in the study of plant pathology and others interested in Scientific endeavours shall be eligible for membership.

(2) Concerns interested in the subject are eligible for being Patrons.

(3) Members may be elected at any regular meeting of the Society or, in the interim between meetings, may be elected by the Council. Application for membership must be endorsed at least by one member.

7. Art. 6 of the Constitution. The second para of this article was amended as follows, after some discussion :—

The Council shall consist of the President, Vice-President, Secretary-Treasurer, and six Councillors. The six Councillors shall represent six Zones, one for each zone. A Zone having less than ten members on October 1st of the year shall have no representative in that year and vacancies will be thrown open for general election. The Councillors shall also serve for one year. The Zones will be as follows :—

Northern Zone.—Punjab, Delhi, Sind, Baluchistan, North Western Frontier Province, and adjoining states.

Mid-Eastern Zone.—United Provinces of Agra and Avadh, Bihar and adjoining States.

Eastern Zone.—Bengal, Assam, Orissa and adjoining States.

Central Zone.—Central Provinces, Hyderabad, Gwalior, Indore and adjoining States.

Western Zone.—Bombay, Baroda, Mysore and adjoining States.

Southern Zone.—Madras, Travancore, Coorg and adjoining States.

8. Rai Bahadur J. C. Luthra was unanimously appointed auditor for 1948.

9. It was resolved that the Lloyd's Bank, New Delhi, be the Bankers of the Indian Phytopathological Society.

10. Three resolutions as suggested by the Lloyd's Bank were passed.

11. It was unanimously resolved that the Secretary-Treasurer and a Councillor residing in the same place as the Secretary-Treasurer, shall jointly operate on the Bank account. In case a Councillor is not available at the same place where the Secretary-Treasurer is stationed, then an ordinary or life-member residing there shall be nominated by the Council to operate on the bank along with the Secretary-Treasurer.

12. Dr. R. S. Vasudeva, Councillor residing in Delhi where the Secretary-Treasurer is stationed, was elected to operate the bank account jointly with the Secretary-Treasurer.

13. It was unanimously resolved that the President be authorised to have the final ballot papers opened and counted, before the Annual General Meeting, by two persons nominated by him, so that the results can be announced at the General Meeting without delay.

INDIAN PHYTOPATHOLOGICAL SOCIETY

CONSTITUTION & BYLAWS

Art. 1. **Name.** The Society shall be known as the "**Indian Phytopathological Society**".

Art. 2. **Aims and Objects.** The aims and objects of the Society shall be:

I. To advance the cause of Mycology and Plant Pathology in India.

II. To encourage and promote mycological and plant pathological study and research in the country.

III. To disseminate the knowledge of Mycology and Plant Pathology.

IV. To facilitate closer association and relations among members and other scientific workers in India and abroad

Art. 3. **Membership.** The Society shall consist of Members and shall include Life-Members, Patrons and Honorary Members. Members joining before 10th January 1948 will be designated Charter Members.

Art. 4. **Dues.** The dues for regular members shall be Rs. 10/- ($3.50 or 15 shillings) a year. Any member may become a Life-Member by paying Rs. 120/- (= $ 42·00 or £ 9) payable either in a lump sum or in instalments within a year from the time a member applies for life-membership. Any one can become a Patron by paying Rs. 1000/- ($ 350 or £ 75), and upon election shall have all the privileges of Members. Ordinary-Members and Life-Members pay Rs. 10/- admission fee, Patrons and foreigners being exempt.

Annual dues shall include subscription to the official organ of the Society and be payable in advance on or before December 20. Bills for dues shall be sent to all Members in October and it will be necessary to discontinue sending the Journal to those whose dues have not been paid by December 20.

Art. 5. (1) All persons interested in the study of plant pathology and others interested in scientific endeavour shall be eligible for membership.

(2) Concerns interested in the subject are eligible for being Patrons.

(3) Members may be elected at any regular meeting of the Society or, in the interim, may be elected by the Council. Application for membership must be endorsed atleast by one member.

Art. 6. **Officers.** The Officers of the Society shall consist of one President, one Vice-President and a Secretary-Treasurer, whose duties shall be those usually peformed by such officers. The President and Vice-President shall serve for one year and the Secretary-Treasurer for three years (or until their successors are elected). Any vacancies occurring in the interim period between elections shall be filled by the Council.

The Council shall consist of the President, Vice-President, Secretary-Treasurer and six Councillors. The six Councillors shall represent six zones, one for each zone.

A zone having less that ten members on October 1st of the year shall have no representation in that year and vacancies will be thrown open for general election. The Councillors shall also serve for one year. The Zones will be as follows :

Northern Zone.—Punjab, Delhi, Sind, Baluchistan, North Western Frontier Province and adjoining States.

Mid-Eastern Zone.—United Provinces of Agra and Avadh, Bihar and adjoining States.

Eastern Zone.—Bengal, Assam, Orissa and adjoining States.

Central Zone.—Central Provinces, Hyderabad, Gwalior, Indore, and adjoining States.

Western Zone.—Bombay, Baroda, Mysore and adjoining States.

Southern Zone.—Madras, Travancore, Coorg and adjoining States.

Art. 7. **Editors & Committees:** The Editors of the official Journal shall be elected by the Council. The President shall appoint all temporary committees that are to serve during his administration and shall fill all vacancies in any of the committees that may occur during the year.

Art. 8. **Election of Officers:** The Secretary-Treasurer shall send to each member of the Society on October 1, a ballot for the nomination of the Officers. If any nominations are lacking, the council shall have the power to make them. The three candidates for each office receiving the highest number of nominating votes shall be placed upon a final ballot to be sent to each Member on December 1. Should the nominating votes received by a candidate place him among the highest three for more than one office, his name shall appear on the final ballot for only the highest office. The officers rank in the order given in article 6. Votes shall be mailed to the Secretary-Treasurer and counted by the Council. A plurality of votes shall elect.

Art. 9. **Meetings.** An annual meeting shall be held at such time and place each year as the Council may select (usually in connection with the Indian Science Congress). An additional meeting for informal discussion and the carrying out of collecting forays may be held in summer or autumn at a time to be selected by the Council. Additional meetings, including special or local meetings for presentation of papers or carrying out of forays, may be arranged by the council at its discretion.

Arts 10. **Divisions.** Branch organizations or units within the Society known as Divisions may be established on a geographical basis provided formal application, setting forth the reasons for the establishment of the Division, is made to the parent body and approved by it.

Art. 11. **Journal.** The Society shall establish a Journal which shall serve as its official organ primarily for the publication of mycological and plant pathological papers by its members (or communicated by members), review of books and literature and for the publication of the report of the Auditing Committee or of other reports, announcements and business of the Society.

Art. 12. **Amendments.** These articles may be amended by a majority vote of the members voting at any regular meeting of the Society, provided that the suggested amendments have been brought to the attention of the Council of the Society in time to be sent to all of the Members at least one month previous to the meeting.

BYLAWS

1. Persons who have gained distinction in Mycology and Plant Pathology shall be eligible for election as Honorary Members, such honorary membership being restricted to foreigners and limited to twenty.

2. When a person has been elected a Member of the Society, the Secretary-Treasurer will inform him of his election and shall send him a copy of the Constitution and Bylaws.

3. No ordinary Member shall be admitted to the privileges of the Society until after the payment of the admission fee and annual subscription.

4. Resignation of membership shall be signified in writing to the Secretary-Treasurer and the Member so resigning shall be liable for the payment of his annual subscription for the current year, together with any arrears up to the date of tendering his resignation.

5. At the annual general meeting, the Secretary-Treasurer shall present his report, together with duly audited accounts ; an auditing committee of two shall be appointed ; Constitution and Bylaws may be revised ; and the election of new office-bearers will be announced. An audited statement shall be annually published in the official organ of the Society.

6. Programmes for annual and other meetings shall be arranged by the Council.

7. The Council may invite any Scientific Society to participate in its Meetings.

INDIAN PHYTOPATHOLOGICAL SOCIETY

Instructions to Authors

Membership in the INDIAN PHYTOPATHOLOGICAL SOCIETY is prerequisite to publishing in INDIAN PHYTOPATHOLOGY but the Editorial Board may relax this rule in the case of contributions of exceptional merit and communicated with a special recommendation by a member. The Editorial Board may invite distinguished scientists to contribute articles of interest to the Society.

Contributions should be on one side of the page, double spaced, with a 1-1/4th inch margin on the left. In form and style, such as punctuation, spelling and use of italics, the manuscript should conform to the best Journals in the U. K. and U.S.A. Authors should strive for a clear and concise style of writing. The name and address of the Institution at which the work was done should be cited. Tables should be numbered and each table should have a heading stating briefly its contents. References to literature should be made as foot notes *only* when four or fewer citations are given. If there are more, they should be listed under 'REFERENCES' at the end of the paper and referred to by date in brackets in the body of the paper. Citations should give the name of the author (or authors), his (or their) initials, year of publication and the full title correctly, followed by the name of the Journal and volume and page numbers. If the title is in a foreign language, then the diacretic signs and capitalization should be as in the original. The names of the Journals should be as abbreviated in the WORLD LIST OF PERIODICALS, 2nd ed., 1934, but as that book may not be available to all, contributors are requested to give the titles in full. Abbreviating will, in that case, be done by the Editors. If an article has not been seen in original, then that should be clearly stated.

Because of high cost of half-tone blocks, carefully made line drawings on Bristol board in black ink will be preferred. Photographs when necessary should be printed on glossy contrast paper and be of best quality. Full page figures and photographs should be made to reduce to 4 x 6 1/2 inches, the standard size for all plates. Each author is allowed one page of half-tone illustration for each article or its equivalent, and the cost of half-tone blocks and paper in excess will be charged to author. Drawings must be drawn to standard scales, so that they can be compared with one another, *e.g.* x10, x50, x100, x250, x500 etc. It is not always possible to get a magnification at a round figure with a camera lucida but the printer can readily reduce drawings at any magnification to the standard, provided a scale is added to the drawing. The scale should measure from 5 to 10 cm. the longer the better and the printer should be instructed to reduce this line to the desired magnification.

Authors are invited to consult Bisby's 'An Introduction to Taxonomy and Nomenclature of Fungi' (1945), pp. 38-41 and Riker's 'The Preparation of manuscripts for Phytopathology,' Phytopathology 36: 953-977, 1946, before preparing their mss and figures.

Articles will be published in the order of their approval for publication but the address of the retiring President and invitation articles will be published when received.

To comply with the International Rules of Botanical Nomanclature, latin descriptions must be supplied to validate new species and genera.

Authors requiring reprints with or without covers should place an order for the copies wanted at the time of returning the proofs and they will be charged actual cost.

INDIAN PHYTOPATHOLOGICAL SOCIETY

COUNCIL FOR 1948

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Vice-President	S. R. Bose
Secretary-Treasurer	B. B. Mundkur (1948-1951)
Councillors	Pushkarnath
	R. S. Vasudeva
	K. C. Mehta
	B. N. Uppal
	G. S. Kulkarni
	K. M. Thomas

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INDIAN PHYTOPATHOLOGY is the official organ of the INDIAN PHYTOPATHOLOGICAL SOCIETY. It is sent free to members in good standing but for others the annual subscription is Rs. 14/- (£1-1-0 or $4.50), post free and payable in advance. There will be, for the present, two issues in a year, comprising about 250 pages but the number of pages and issues per annum will be gradually increased. Subscriptions should be sent to the Secretary-Treasurer, INDIAN PHYTOPATHOLOGICAL SOCIETY, Pusa Buildings, New Delhi.

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